STUDIES ON THE DISEASES OF WHEAT IN NEW ZEALAND

CAUSED BY *SEPTORIA* AND *TILLETIA* SPP.

A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE

OF

MASTER OF AGRICULTURAL SCIENCE

IN THE

UNIVERSITY OF CANTERBURY

BY

J.G. HAMPTON

LINCOLN COLLEGE

1975

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ABSTRACT

The literature on the pathogens *Septoria tritici* and *Septoria nodorum*, and the wheat diseases they cause has been reviewed, including methods of disease assessment and some aspects of fungicide application and mode of action.

Both *S. tritici* and *S. nodorum* have ascogenous states, and these are a *Mycosphaerella* sp. and *Leptosphaeria nodorum* respectively. Perithecia of both were found in old leaf tissue in wheat stubble soon after harvest. Perithecia began to mature by the end of February, and wind-borne ascospores were trapped from March to June, 1974. Those of *L. nodorum* were trapped two weeks before any *Mycosphaerella* ascospores were found. The release of both ascospore types appeared to be in response to moisture. Viable ascospores of both types could still be detected in June. Ascospores of these two species would be capable of initiating infection in autumn-sown wheat crops some distance from other inoculum sources (i.e. pycnidia), and hence would be important in the epidemiology of the *Septoria* diseases of wheat.

A field survey of wheat crops in Canterbury during 1973 failed to detect *S. nodorum*. Symptoms caused by this fungus were first seen on the basal leaves of volunteer wheat plants in spring of 1974, as discrete brown necrotic lesions, bordered by a ring of chlorotic tissue. The lesions did not coalesce, as do those caused by *S. tritici*. At no stage did pycnidia develop on leaves in the field, but when leaves with lesions

were floated on a benzimidazole solution and placed under near ultra-violet (NUV) light for a 12 hour photoperiod, pycnidia developed four days later. Laboratory tests showed that a temperature of 18-24°C, light, 95-100% R.H. and senescing tissue were important factors governing the appearance of pycnidia of *S. nodorum* in leaf tissue. *S. nodorum* was isolated from these pycnidia, and inoculation experiments showed that the cultures of this fungus were pathogenic to wheat. Cultures which developed from ascospores of *L. nodorum* were found to be *S. nodorum* and also shown to be pathogenic to wheat. Colonies of *S. nodorum* grew on malt, but better on oatmeal and potato-dextrose agar. Sporulation occurred after 20-30 days incubation at 20-22°C under a light regime of 12 hours NUV/12 hours darkness, and was greatest in those colonies grown on oatmeal agar. *S. nodorum* was not observed on the glumes of wheat. An examination of twenty-five Canterbury lines of wheat seed failed to reveal the presence of *S. nodorum*.

S. tritici was common in wheat crops in Canterbury during 1973 and 1974. Symptoms first appeared on young leaves as discrete chlorotic lesions, which eventually coalesced into large necrotic lesions, containing black pycnidia, often with a broad chlorotic marginal zone. These lesions often covered the entire leaf area. By heading (G.S. 10), *S. tritici* infection could be found on all leaves of the wheat plant, but was not observed on the glumes. A 'black' and a 'white' cultural race of *S. tritici* was isolated from separate lesions

on wheat plants from the field. These races, inoculated to wheat, produced identical symptoms and signs in the host plant, but in culture were distinct and did not produce any variants. Both could be isolated from lesions on leaves throughout the growing season of the plant, but at no stage in time were both races isolated from the same lesion. Pycnidia of *S. tritici* containing pycnidiospores, were also found to be present within old leaves of wheat stubble from February to June. Pycnidiospores from these pycnidia were still viable in May, but by June, their viability had markedly decreased. Aotea wheat plants in a replicated field trial became naturally infected by *S. tritici*. When sprayed with benomyl or mancozeb at ear emergence, a significant increase in 1000 grain weight was produced. This increase appeared to be due to a reduction of infection and delaying of senescence of the flag leaf. However, yield was not significantly increased, although there was 14% more grain produced from treated plots. Benomyl and thiophanate-methyl applied at tillering did not increase yield, although *S. tritici* was controlled for up to six weeks.

Field assessments of six wheat varieties showed that Hilgendorf and Kopara showed some tolerance to *S. tritici*, Arawa and Karamu were moderately susceptible, and Aotea and Gamenya were highly susceptible. When inoculated with *S. tritici* in the laboratory, these varieties reacted similarly. Varietal reaction to *S. nodorum* in the laboratory was similar to the *S. tritici* results.

The literature on stinking smut, or bunt, (*Tilletia caries*, and *Tilletia foetida*) of wheat in New Zealand was reviewed, including data on incidence, control measures, and the assessment of fungicidal activity on seed by bioassay methods.

Grain samples of eight wheat varieties from each of the 1970 to 1973 harvests were tested by means of a 'Washings test' for the presence of *Tilletia* teliospores. The main variety in New Zealand, Aotea, contained significantly more teliospores per sample than any other variety. Further Aotea samples were tested to indicate the incidence of bunt in the 1973 crop. A total of 244 Aotea wheat samples from the wheat growing counties of New Zealand were tested for *Tilletia* teliospores, with the number of samples tested per county being proportional to the area of wheat grown. Of these, 2 samples from the North Island, 6 from North Canterbury, 4 from Mid-Canterbury, 6 from South Canterbury and 4 from Southland were infected; the 22 infected samples being 9% of the total number tested. All teliospores detected were those of *Tilletia caries*, and all were present as trace amounts.

A visual examination of 21 treated wheat seed samples from five grain merchants in Canterbury showed that the coverage of seed with fungicide, applied as a slurry, was very variable. This was confirmed by bioassay tests on nine samples which showed that nearly 30% of the seeds tested were receiving less than half the recommended rate of fungicide coverage, and approximately 6% of these seeds were receiving no fungicide at all. This appeared

to be a fault of the slurry treating machine used, rather than that of the operators. Three samples of wheat seed, slurry treated by another type of machine, were found to be less variable in coverage.

PREFACE

New Zealand wheat crops are attacked by a number of fungal pathogens. The severity of the diseases varies with environmental conditions, but either directly or indirectly, losses in yield occur (Blair, 1972).

This thesis is concerned with two disease problems of wheat. The first is that of the *Septoria* diseases of wheat caused by *Septoria tritici* Rob. ex Desm., and *Septoria nodorum* (Berk.) Berk. in Berk. and Br. In New Zealand little is known about these two pathogens, and the associated diseases. This study investigated the life cycle of the fungi, their effects on wheat yield, and methods of control.

The second problem is the stinking smuts or bunts of wheat caused by *Tilletia caries* (DC)Tul., and *Tilletia foetida* (Wallr.) Liro. These pathogens have been responsible in the past for large wheat losses (Blair, 1956), but are now controlled by the application of chemicals to the wheat seed on which spores of the fungi are carried (Sanderson, 1964a). The incidence of these fungi in New Zealand wheat crops was determined, and the efficiency of seed-dressing techniques was also investigated.

PART A. SEPTORIA DISEASES OF WHEAT

SECTION A 1: LITERATURE REVIEW

C H A P T E R I

SEPTORIA FUNGI CAUSING WHEAT DISEASES

1.1 BIOLOGY OF THE SEPTORIA FUNGI

1.11 Introduction

Septoria diseases of wheat are of common occurrence and are caused by *Septoria nodorum* (Berk.) Berk. in Berk. and Br., perfect state *Leptosphaeria nodorum* Muller; and *Septoria tritici* Rob. ex Desm., perfect state *Mycosphaerella sp.* (Sanderson, 1972 a). Under suitable conditions, these pathogens may cause marked symptoms, but at other times the symptoms may be relatively inconspicuous. Nevertheless, the losses may be substantial even when the disease level is moderate. (Shipton, 1968).

In Canada (Johnson, 1947; Connors, 1956) and the North Central United States (Hosford, Hogenson, Hugguelet and Kiesling 1969) *Septoria avenae* Frank f. sp. *triticea* T Johnson, perfect state *Leptosphaeria avenaria* Weber f. sp. *triticea* T Johnson, has been reported as common on wheat, where it causes severe foliage damage to some spring wheat cultivars. As yet this species has not been recorded on wheat in New Zealand.

1.12 Taxonomy and Nomenclature

Berkeley (1845) described the fungus *Depazea nodorum* on the discoloured nodes of wheat in England. Later, Berkeley (Berkeley and Broome, 1850) provided a Latin description but referred the specimen previously described to the genus *Septoria*. Under article 46 of the Code of Botanical Nomenclature (Lanjouw, 1966) the citation now should be *Septoria nodorum* (Berk.) Berk. in Berk. and Br. (Shipton, Boyd, Rosielle and Shearer, 1971).

Voglino (1904) found perithecia in cultures of *S. nodorum*, and identified the perfect state as *Sphaerella exitialis* Mor., (Shipton et al, 1971). Weber (1922) thought that the perfect state would be better placed in the genus *Leptosphaeria*. However it was not until 1952 that Müller (1952) described *L. nodorum* and established it as the perfect state of *S. nodorum*.

Desmazieres (1842) published a description of *Septoria tritici* which he attributed to Roberge. There is some confusion as to who proposed the name *Septoria tritici*, but Shipton et al. (1971) accredited it to Roberge, and consider the correct citation to be *S. tritici* Rob. ex Desm.

The perfect state of *S. tritici* remained undiscovered until Sanderson (1972a, 1974) found it to be a *Mycosphaerella* sp., possibly *Mycosphaerella graminicola*, which Cooke (1892) named *Sphaerella graminicola*. A Latin description of this organism ex wheat has yet to be published.

There was a tendency to treat *S. tritici* Rob. ex Desm. and *S. graminum* Desm. as synonyms (Weber, 1922), but Sprague (1938) clarified the position of *S. graminum* and indicated that it only occurred on grasses.

1.13 Morphology

a) *Septoria nodorum* (Berk.) Berk. in Berk. and Br.

The morphology of *S. nodorum* has been described by Anon (1966) as:

"Pycnidia immersed, globose, honey brown, becoming darker, epiphyllous, 140-200 µm diam., wall up to 5 cells thick composed of thin-walled, honey yellow, pseudoparenchymatic cells somewhat larger and thicker walled near the ostiole which is slightly papillate and measures up to 25 µm diam. Conidia hyaline, cylindrical, straight, sometimes irregularly curved, mostly three septate, apex and base obtuse, 22-30 x 2.5 µm, formed as phialospores from the shortly obpyriform, hyaline, aseptate, undifferentiated inner cells of the pycnidial wall, 4-6 x 6 µm.

Ascocarps immersed, globose, finally depressed, mid-brown to black, 150-200 µm diameter. Asci are clavate, cylindrical or curved, shortly stipitate, 8-spored, 47.5-65 x 8-10 µm; ascus wall thick bitunicate. Ascospores fusoid, subhyaline to pale brown, 3-septate, constricted at the septa, penultimate cell swollen, 19.5-22.5 x 4 µm. Pseudoparaphyses filiform, hyaline, septate."

b) *Septoria tritici* Rob ex. Desm.

The morphology of *S. tritici* has been described by Anon (1966) as: "Pycnidia immersed, globose to elliptical, honey brown, turning black at maturity, amphigenous, often aggregated and arranged longitudinally between the veins, 80-150 µm diam., wall up to 8 cells thick, composed of thick walled, dark brown, almost sclerotoid pseudo-parenchymatic cells, darker near the ostiole which is neither papillate nor protruding 10-20 µm diam. Conidia hyaline, filiform, 2-3 septate, typically curved, gradually tapered to an acute apex, base obtuse, 43-70 x 1.5-2 µm, formed as phialospores from narrowly obpyriform, rarely septate, hyaline conidiophores, measuring 10-20 x 4-5 µm."

Sanderson (1972a) described the perithecia as: single, globose, becoming laterally compressed, 76-80 x 77-100 µm; dark brown. Superficially immersed in dead leaf blade tissue of the wheat stubble; poorly developed stroma present. Perithecial wall 2-layered, cell walls of outer layer thickened and pigmented; asci bitunicate, obpyriform, 34-41 x 11-13 µm, developing from the basal tissue of the perithecia; no paraphyses; 8 ascospores, either biseriate or irregularly arranged, 2 celled, hyaline, elliptical, with one cell tending to be slightly larger; 10-15 x 2.5-3 µm.

1.14 Symptoms and their Development

a) *Septoria nodorum*

Weber (1922) first described germination of *S. nodorum* and reported that germ tubes penetrated directly. Shipton et al. (1971), noted that stomatal entry has not been observed. On reaching the mesophyl cells, the penetration hyphae branched and ramified intercullularly, but did not produce haustoria. Cell degeneration occurred when the intercellular spaces were filled with hyphae.

On the leaves, irregular shaped lesions developed, with the centre light-brown in colour, and surrounded by a medium to dark-brown margin. The culms, nodes and rachis may be almost entirely covered by elongated, diffuse, coalescing lesions (Shipton et al., 1971). Infected tissues may take on a light yellowish-brown, dark brown or almost black colouration. Pycnidia formed within 10-21 days (Weber, 1922; Scharen, 1964).

Infected glumes and awns at first showed irregular brownish lesions which later turned a dark brown to almost black, on the upper areas of this tissue.

Seeds may become infected, but it is not always possible to determine visually whether this is so. With severe infection, large areas may be covered with irregular light to dark brown coalescing lesions (Shipton et al., 1971).

b) *Septoria tritici*

Pycnidiospores were able to germinate in water in 4-36 hours, and entry by direct penetration or through stomata occurred within 24 hours (Weber, 1922; Luthra, Sattar and Ghani, 1938). After penetration, hyphae enlarged slightly and grew intercellularly. Haustoria were not produced. Elongated lesions formed in which pycnidia developed. Pycnidia were situated in the substomatal cavities with their ostioles directly under the stomates (Shipton et al., 1971).

Infection of leaves and culms was evident in 6-36 days with the appearance of light green chlorotic areas which developed into elongated, yellowish to light brown necrotic lesions (Weber, 1922; Hilu, 1956). The pathogen is also capable of causing symptoms on the rachis and glumes under field conditions (Jones and Cooke, 1969), and infection may extend to the awns (Luthra et al., 1938; Jones and Cooke, 1969).

1.15 Life Cycle

a) *Septoria nodorum*

Reports by Weber (1922) and Müller (1952) indicated that the perfect state has not been observed very frequently, and Shipton et al. (1971) considered that it must play a minor role in epidemiology. Infected plant debris may serve as an inoculum source (Weber, 1922), for pycnidiospores were found to overwinter within the pycnidia on infected straw with little or no loss of viability, and 30% viable spores could be recovered after 18 months storage out of doors.

Scharen (1964) found that after wetting of the straw, new pycnidia were produced, and during drying of this straw, there was a replenishment of spores in older fruiting bodies. When infected material was subjected to alternate wetting and drying cycles, it was capable of producing spores for as many as eight cycles (Shipton et al., 1971).

Spore release occurred from pycnidia under moist conditions (Weber, 1922), or conditions of high humidity. Scharen (1966) found that under artificial conditions, spore exudation commenced soon after wetting and continued unabated for 3 hours, then steadily diminished until it ceased after 6 to 7 hours.

Pycnidia produced in the leaves of a host matured in 10 to 21 days, and the pycnidiospores initiated secondary infection (Scharen, 1966). Using simulated wind and rain conditions Scharen (1966) demonstrated infection of nearby plants by inoculum from already infected leaves. The lower leaves were first infected, and the fungus spread up the plant in an orderly manner. Lower leaf lesions probably acted as an inoculum source for higher leaves. Shipton et al. (1971) reported that the distance over which the pycnidial inoculum may be carried by wind is unknown.

Shipton et al. (1971) consider that inoculum carried on straw of cereals and grasses is perhaps the major source of infection, but that seed-borne inoculum is of importance. Hewitt (1965) believed that it must be an

important inoculum source in Great Britain and other reports (Shaw and Valder, 1952; Noble, 1956; Kietreiber, 1961, 1967a; Shipton and Chambers, 1966), indicate its importance in other areas. The role of seed-borne inoculum will depend on its longevity in and on the seed, as well as the efficiency of seed treatments. Work by Shipton (Shipton et al., 1971) showed that about two thirds of infected seed was free from *S. nodorum* after seven to nine months storage.

b) *Septoria tritici*

The sexual stage has, as yet, only been recorded in New Zealand (Sanderson, 1972a). Consequently, its importance in the life cycle is not as yet known, but Sanderson (1974) has shown that the perithecia of the fungus on wheat stubble may act as a good primary source of ascospores for infection of Autumn-sown wheat.

Weber (1922) noted that after rain, pycnidiospores were exuded in great numbers from overwintered pycnidia on dead leaves. Viability of the material seemed to be dependent on the conditions under which pycnidia were produced and stored. In the field, Hilu and Bever (1957) showed that viability was rapidly lost after 3 months; however, in New Zealand, Wenham (1959) found that after 5 months, viability was rapidly lost. In the laboratory, Hilu and Bever (1957) and Luthra et al. (1938) found survival better at lower temperatures (5-15^{o}C).

Weber (1922), Luthra et al. (1938), Hilu and Bever (1957) and Wenham (1959) found that inoculum produced in one season was available to initiate infection in the next, and volunteer infected plants may have played a role in the survival and production of inoculum, (Weber, 1922; Wenham, 1959).

Windblown debris and pycnidia aid in the dispersal of inoculum, (Weber, 1922). Pycnidiospore dispersal can also occur by means of splash dispersal, but the distance over which this takes place is unknown. Luthra et al. (1938) found that spores retained their viability in dry conditions in the summer for 14 days. Therefore, infection of plants some distance from the source of inoculum could well occur.

1.16 Factors affecting Infection and Symptom Expression

a) Humidity, temperature and light

Moisture is of prime importance for the release and dispersal of spores of *S. nodorum* (Weber, 1922; Scharen, 1966), and humid conditions are usually needed for infection (Scharen, 1964). Spore germination is favoured in general by temperatures between 10-28°C (Thomas, 1962).

Seed infection by *S. nodorum* was favoured by wet periods at heading (Hewitt, 1965). Infection of seedlings from seed-borne inoculum depended largely on the speed of coleoptile elongation, for conditions favouring rapid elongation did not favour seedling infection, (Kietreiber 1961, 1967b). Infection was greater at 10°C than at 20°C.

S. tritici also needed wet weather for spore release and infection (Weber, 1922; Luthra et al, 1938). Spores were found to germinate within 4-36 hours and infection occurred within 24 hours (Weber, 1922; Luthra et al, 1938; Hilu and Bever, 1957). A temperature of 22-24°C favoured germination (Weber, 1922), while infection was favoured by temperatures between 16-21°C.

Symptom expression was dependent on the length of the humid period for both *S. tritici* and *S. nodorum* (Shearer, 1967). In both cases the longer the humid period after infection, up to a maximum of 96 hours, the greater the symtom development. The effect of light on symptom expression is not known (Shipton et al., 1971).

b) Spore Load

With both *S. nodorum* and *S. tritici*, an increase in the concentration of spores in the inoculum lead to a greater amount of infection (Rosielle, 1968; Cooke and Jones, 1970). With *S. tritici*, high spore concentrations lead to the early appearance of symptoms and sporulation (Shipton et al. 1971).

c) Plant Nutrition

Thomas (1962) found that high levels of nitrogen predisposed wheat seedlings to *S. nodorum* infection. This probably also applies to *S. tritici* (Fellows, 1962). The role of other elements in the susceptibility of wheat plants to *Septoria* diseases is still largely unknown (Shipton et al., 1971).

d) Plant Age

Jones and Odebunmi (1971) found that when wheat at different growth stages (Large, 1954), was artificially inoculated with *S. nodorum*, there were significantly less symptoms of disease following inoculation at growth stage (G.S.) 3, than at G.S. 6, 10 and 10.5. This suggests that the younger wheat tissues were more resistant to attack by *S. nodorum*. Other reports are conflicting (Shipton et al., 1971).

e) Interaction with Other Diseases

Plants already infected by one pathogen may be either more susceptible or more resistant to infection by a second, unrelated pathogen (Yarwood, 1959). Other leaf and root pathogens may be associated with *Septoria* infections, but Shipton et al. (1971) could find no conclusive evidence that there were interactions causing an increase or decrease in susceptibility.

1.17 Host Range and Pathogenic Specialization

a) *Septoria nodorum*

Although *S. nodorum* has been isolated from cereals and grasses belonging to a number of genera (Weber, 1922; Sprague, 1950, 1955; Williams and Jones, 1973), strict host specialization has not been demonstrated (Shipton et al, 1971). Rosielle (1968) and Thomas (1962) showed that there were slight variations in the pathogenicity of isolates on particular wheat varieties, but Shipton et al. (1971) suggest that in both cases, it would be difficult to claim that this variability constituted evidence of physiologic specialization.

b) *Septoria tritici*

The pathogen has been recorded on fifteen genera of grasses (Sprague, 1950) but not all these may be able to act as alternate hosts for the form on wheat.

According to Sprague (1950), three specialized forms, in addition to that on wheat, can be separated: *Septoria tritici* f.sp. *avenae*, found only on *Avena byzantina* K. Koch, *A. fatua* L. and *A. sativa* L. (Sprague, 1934); *Septoria tritici* f.sp. *holci*, specific to *Holcus* (Sprague, 1944); *Septoria tritici* f.sp. *lolicola*, apparently restricted to *Lolium multiflorum*, and *L. perenne* (Sprague, 1944), although Latch (1966) obtained infection of *Agropyron repens* (L.) Beauv. under artificial conditions. Work by Morales (1958) and Shearer (unpublished data) failed to reveal any evidence of physiologic specialization.

1.2 INCIDENCE OF SEPTORIA IN NEW ZEALAND

The first possible reference to *Septoria* diseases of wheat in New Zealand was made by Cunningham (1927), when in a report of a disease survey made in January of that year he referred to 'wheat spot and oat spot, *Septoria graminum*'. It was not until 1948 that Blair (1948) in reporting work at Lincoln College noted that *Septoria tritici* had been isolated from diseased wheat tissue by H.T. Wenham. Wenham (1948) noted that Canterbury wheat crops had a high percentage infection of *S. tritici* in the 1947 and 1948 seasons and suggested that the increase in incidence of the disease could be due to previous misinterpretation of the typical field symptom

(spring yellows) as a non-parasitic reaction, or incorrect identification of pycnidia of *S. tritici* as perithecia of *Erysiphe graminis*. Of these two explanations, the former seems more probable.

Since the disease was recognised, outbreaks have been reported in Canterbury wheat crops nearly every season (Cruickshank, 1953; Anon., 1956; Wright, 1959; Anon., 1964; Smith and Wright, 1964; Anon., 1967). These reports showed that *Septoria* blotch together with powdery mildew and leaf rust, were present in a large percent of wheat crops. These diseases occurred every season, although there were seasonal fluctuations in their incidence. The majority of reports refer to leaf blotch caused by *S. tritici*. However, *S. nodorum* can cause similar symptoms on leaf tissue. There is a record (Anon., 1953) of *S. nodorum* being associated with *S. tritici* in causing severe leaf infection in the 1953 Canterbury wheat crop, but no evidence was presented to confirm its identification. Wenham (1959) stated that *S. nodorum* had not been recorded in New Zealand. However Sanderson (1974) has found pycnidiospores of *S. nodorum* on diseased wheat tissue from Canterbury and Southland, and has listed it in his description and key of wheat diseases in New Zealand (Sanderson, 1972b).

1.3 LOSSES DUE TO SEPTORIA DISEASES

Shipton et al. (1971) stated that there have not been enough critical studies to establish the losses attributable to *Septoria* diseases. Both *S. tritici* and *S. nodorum* occur in all the major wheat producing countries, and reports of losses in grain yield and quality have come from most, although unfortunately, many are not precise, and simply state 'considerable', 'severe', etc.

Overseas reports have shown losses from natural infection of *S. tritici* from 28% (Caldwell and Narvaes, 1960) to 60-70% in early sown wheat (Haskell, 1928). Artificial inoculation of plants with *S. tritici* produced yield losses of up to 45% (Caldwell and Narvaes, 1960) and 24% (Jones and Odebunmi, 1971). Losses of 10% (Haskell and Wood, 1928) and 7-27% (Kobel, 1956) have been reported from natural infections of *S. nodorum*, while Jones and Odebunmi (1971) reported yield reductions of 40%, and Brönninmann (1968b), losses of up to 65% with artificially-induced epidemics. Jenkins and Morgan (1969) found that the combined effect of *S. tritici* and *S. nodorum* produced losses of 26%.

The only New Zealand work on the effect of *Septoria* diseases on yield has been by Sanderson (1964b), who found a 10% increase in yield from only partial control of *S. tritici*, and then estimated that the total loss from *Septoria* in wheat was around 20%.

1.31 Stage of Plant Development for Infection Related to Losses

Septoria diseases can be a major limiting factor to crop production in two main phases of the plant's development; the tillering phase (G.S. 1-5)(Bensuade, 1929), and ear emergence (G.S. 10-10.5)(Brönnimann, 1968a and b).

a) Tillering phase

S. tritici and *S. nodorum* differ in that *S. nodorum* is seed-borne and hence can affect the wheat plant during germination. Hewett (1965), Shipton and Chambers (1966) and others have found that severe seed infection by *S. nodorum* may impair seed germination. Lesions may develop on coleoptiles and the seedlings may not survive (Noble, 1956). Severe seedling infection by *S. nodorum* may reduce tiller production (Bensuade, 1929).

Symptoms of *S. tritici* can be found first at the one to three leaf stage (G.S. 1-3), when yellow-brown necrotic leaf lesions, yellowed leaf tips, and sometimes black pycnidia can be seen (Wenham, 1949). The activity of the pathogen at this stage can have the effect of causing the premature senescence of either the whole or large parts of the leaf, or predispose the leaf to either attack by other pathogens or the effects of adverse climatic conditions. Wenham (1949) suggested that a severe attack at this stage would cause most damage to the crop, because of the resultant tiller death.

b) Ear emergence

Both pathogens can, and often do, infect at all growth stages of the wheat plant, but at ear emergence, severe infection by the pathogen will be most likely to have a direct effect on yields. Grain development has been shown to be primarily a function of that portion of the plant above the flag leaf node and the time that part can remain photosynthetically active (Thorne, 1965, 1966; Rawson and Hofstra, 1969). Fungal attack of these parts of the plant will cause yield losses, mainly because they decrease the photosynthetic area, and hence grain weight, (Brönnimann, 1968a and b). Because *S. nodorum* is more common in Great Britain in the head and flag leaf than *S. tritici*, the former could be expected to reduce yields to a greater extent than the latter, and this is supported by the results of Jones and Odebunmi (1971). The *Septoria* diseases may affect the economic value of their hosts at these two main stages, but the relative importance of infection at other stages on subsequent development has not been adequately investigated (Shipton et al., 1971).

1.32 Factors Responsible for Losses by *Septoria*

Yield reductions as a result of *Septoria* infection occur mainly through decreases in grain weight and numbers of grains per plant, (Brönnimann, 1968a and b). It would seem therefore, that the effect of the pathogen on the physiology of the host is to reduce the supply of carbohydrate to the ear.

Manners (1969), and Smith, Muscatine and Lewis (1969) believed that this was the case with losses of yield caused by rusts in wheat. Similar studies have not yet been completed with *Septoria*, but it is reasonable to assume that the mechanisms responsible for reducing the supply of carbohydrate to the ear are similar to those operating for the rust fungi: viz. decrease in host assimilation; increase in host respiration; and the fungus acting as a sink through its own growth and metabolism (Manners and Myers, 1973).

a) Effects on assimilation

Certain effects on the photosynthetic system may be readily observed. The initial *Septoria* lesions cause the whole leaf to become chlorotic. The chlorophyl content of the leaf is reduced by infection, due to destruction of chloroplasts. However, with mildew- (*Erysiphe graminis*) infected wheat leaves, Allen (1942) found that the photosynthetic activity of the chloroplasts remaining did not decline.

Scharen and Taylor (1968), working with *S. nodorum* infected wheat tissue, found that net CO_2 assimilation by heads, peduncles, and flag leaves was reduced 27%, while Scharen and Krupinsky (1969) found a reduction in photosynthesis from 14-46% with different wheat varieties. These results were then correlated with seed yields and the yield losses were always greater than the reductions in photosynthesis (as measured by CO_2 absorption). This difference was probably due to interference by the pathogen with translocation of assimilates.

b) Effect on respiration

Increases in respiration of wheat leaves infected by *E. graminus* have been reported (Allen, 1942), and also for other pathogenic fungi (Farkas and Kiraly, 1955; Shaw, 1963). The initial increase in respiration occurred with the appearance of visible symptoms, rose to a maximal rate coincident with sporulation, and declined after this stage, (Goodman, Kiraly and Zaitlin, 1967).

The mechanism of the respiratory increase in diseased plants is not clear. Goodman et al. (1967), suggested two possibilities:

(i) Uncoupling of oxidative phosphorylation, which could cause increased oxygen uptake. As a result of uncoupling a high level of ADP (phosphate accepter) accumulates, which fosters an increased rate in respiration.

(ii) The accumulation of metabolites around lesions (Crowdy and Manners, 1971) which could lead to an increased consumption of ATP in synthetic processes, and the resultant increases in ADP would lead to the production of a higher rate of respiration.

Scharen and Taylor (1968) with *S. nodorum*, found little difference in respiration between healthy and infected plant leaves and ears. However, Scheffer (1960), working with tomato plants infected with *Fusarium oxysporum* Schlect.f. *lycopersici* (Sacc.) Snyd. and Hans., found that respiratory

patterns were dependent upon conditions of plant growth. Oxygen uptake by leaves of infected plants grown on low nitrogen was higher than that of comparable non-infected plants. Plants grown on high nitrogen showed no difference between infected and non-infected leaves as regards oxygen uptake. As Scharen and Taylor (1968) grew their plants under high nitrogen conditions, this may explain their lack of difference between control and infected plants.

c) Effect on transport

With obligate fungal pathogens, a consequence of infection is the accumulation of materials at the infection site. (Gottlieb and Garner, 1946; Shaw and Samborski, 1956; Dodson, Manners and Myers, 1965). Much of the accumulation is in the fungal tissues rather than in the host. However, with facultative pathogens, accumulation of nutrients around infection courts does not occur (Goodman et al., 1967).

Scharen and Taylor (1968) interpreted the difference between yield reduction and reduced photosynthesis (after attack by *S. nodorum*), as the diversion of photosynthate from normal translocation pathways, and calculated that about 20% of the photosynthate produced was diverted in this way. Livne and Daly (1966) showed that rusted bean leaves interfered with normal translocation of photosynthetic products by 'pumping' them to infection sites that become alternate 'sinks'. Thrower and Thrower (1966) also demonstrated this with *Uromyces fabae* on broad beans. However, Doodson et al. (1965) were unable to demonstrate this with comparable

experiments with yellow rust (*Puccinia striiformis*), on wheat. Scharen and Taylor (1968) suggested that the diversion from normal translocation pathways of photosynthate did occur as a result of *S. nodorum* infection, but presented no evidence to support this. It is obvious that further work is needed to determine whether *S. nodorum* infection sites do act as an alternative 'sink', and hence disrupt the normal photosynthate flow.

1.4 CONTROL OF SEPTORIA DISEASES

Shipton et al. (1971) suggested the following methods for control of *Septoria* diseases of wheat: resistant varieties; seed treatments; cultural practices; foliar sprays; quarantine.

1.41 Breeding of Resistant Varieties

Heritable resistance to *S. tritici*, usually as a single dominant or recessive gene, has been clearly demonstrated (Mackie, 1929; Rosen, 1947; Morales, 1958; Rillo, 1968). Shipton et al. (1971) concluded that although there were a number of other reports on resistance in the literature, they were of questionable value, as the importance of the conditions governing infection and symptom expression may not have been fully appreciated. The use of resistant varieties is likely to be the most effective way to control the disease, but as yet, there has been little progress in this field.

The use of resistant varieties for control of *S. nodorum* could also be of value, but there have been no convincing demonstrations that resistance was available or that the tolerance observed was heritable (Shipton et al., 1971).

1.42 Seed Treatments

Noble et al. (1958), and Hewett (1965) showed that *S. nodorum* is frequently seed-borne. Noble et al. (1958) also reported that *S. tritici* was seed-borne, but Shipton et al. (1971) stated that seed-borne infection of *S. tritici* has not been demonstrated.

Organic mercurials have been used for effective control of *S. nodorum* (Burhardt, 1954; Kietreiber, 1967b; Schuhmann, 1967). Zineb, maneb, dichlone and chloranil were effective against *S. nodorum* in France (Shipton et al., 1971), while triarimol and benomyl reduced infection of seedlings, but were inferior to mercury (Brooks, 1972). Thiabendazole was found to be effective and comparable to maneb and mercury (Darpoux, Ventura and Cassini, 1968).

Sanderson (1974) treated wheat seed with benomyl and found this controlled *S. tritici* for up to six weeks after emergence. In this case the systemic fungicide presumably moved via the roots to the young tillers, where it protected the leaves from infection by air-borne ascospores. However, after this time, the disease became established.

Although seed treatment for control of *S. nodorum* can prevent establishment of the disease at emergence and tillering, there are other inoculum sources, such as wind-borne ascospores, and splash dispersed pycnidiospores which can infect the crop at later stages of development. If no attempt is made to control this alternate inoculum, the value of applying fungicide to seed for control of the disease may be minimal.

1.43 Cultural Practices

A number of cultural practices have been recommended for the control of *Septoria* diseases. Crop rotation has been advocated by Dickson (1956) and Feekes (1967). This method would be effective if only pycnidia and pycnidiospores were involved. It is now of less value as the importance of wind-borne ascospores of both species is being realised, as these could be blown in from surrounding crops. Removal of stubble, either by deep ploughing or burning, can reduce the incidence of infection in suceeding crops. Wenham (1959) advocated this for New Zealand conditions, as did Luthra et al. (1938) for control in India. In Europe or U.S.A. this method has not been recommended, perhaps because the role of the stubble as a primary source of inoculum has not been determined.

Both Weber (1922) and Wenham (1959) showed that volunteer wheat can be important as an inoculum source. Williams and Jones (1973) showed that both fungi can survive on grass hosts, and form pycnidia on senescent tissue, but

whether this plays a part in the epidemiology of the fungi is not known. However, removal of both volunteer wheat plants and associated grasses could give some measure of control.

Jones and Williams (1971) found that the herbicide paraquat will inhibit growth of *S. tritici* and *S. nodorum* in culture. It was suggested that stubble treated with paraquat may have a reduced capacity for producing and supporting overwintering inoculum.

The use of early or late maturing varieties, and alterations in sowing date have been advocated (Doling, 1961), but Shipton et al. (1971) suggested that this may not be very effective as inoculum seems to be present through all stages of the growing season.

1.44 Foliar Sprays

This aspect of disease control has received little attention although reduction in the level of infection by regular spraying of leaves is possible. Sanderson (1964b), using captan and zineb sprays at fortnightly intervals, found reductions in incidence of *S. tritici* of 14.5% and 35% respectively. Neither fungicide completely controlled the disease and neither was an economic proposition. Shipton (1968) used sprays of zineb and maneb at weekly intervals commencing at either the five-leaf stage or at anthesis (G.S. 10.5.2), and found that the level of infection was reduced, more so with maneb than zineb. Jenkins and Morgan (1969) reported a partial control of *Septoria* diseases with frequent application of tank-mix zineb, with the full spray programme increasing yields by some 35%.

Although *S. nodorum* can be controlled by seed treatment, Brookes (1972) reported good control with programmes of benomyl sprays, and Hampel and Pommer (1972) reported that application of a benzimidazole derivative (BAS3460F) to wheat at ear emergence increased yields by 7-10%.

At present, spraying for *Septoria* control alone may not be economic, but with the systemic fungicides now available it is likely that one fungicide will give effective control of a number of leaf and head pathogens, including *Septoria*.

1.45 Plant Quarantine

As far as is known, plant quarantine has never been used to prevent spread of wheat diseases caused by *Septoria* (Shipton et al., 1971). The ability of inoculum to persist in seed and straw for lengthy periods suggests that spread does occur in this way, and Pollock (1945) reported that Australian wheat at U.S. ports contained *S. tritici* on the straw. However, *Septoria* diseases occur wherever wheat is grown on a large scale, so that quarantine measures are not likely to be considered necessary, unless new virulent strains appear in specific areas.

C H A P T E R II

METHODS OF DISEASE ASSESSMENT

A plant is healthy or normal, when it can carry out its physiological functions to the best of its genetic potential. Whenever one or more of these functions is interfered with beyond a certain deviation from the normal, then the plant becomes diseased (Agrios, 1970). The primary causes of diseases are either pathogens or environmental factors, but the way in which they manifest themselves varies considerably with the causal agent, and sometimes with the plant.

The main reason for measuring plant diseases is to obtain quantitative data on the occurrence and development of disease. Such data can be used to: assess the relative importance of different diseases by comparing their incidence and severity in agricultural crops; to determine the relationship between disease severity and yield loss in the crop; and to distinguish treatment differences that cannot be detected by measurement of yield or quality. For example, disease measurement can test the relative efficiency of fungicides and their respective formulations, and in variety trials can detect small differences in disease resistance between varieties (James, 1971).

According to Moore (1969), the three basic requirements for field assessment methods are as follows:

a) To enable reasonably accurate assessments of disease incidence to be recorded.

b) To be repeatable so that different people will make similar assessments.

c) Whenever possible, to provide a basis on which estimates of loss can be made.

Each disease and host plant present different problems. Large (1966) discussed the factors involved in the choice and application of the most suitable and readily available methods. Recording of plant diseases can be simply by counting the number of infected plants and expressing this as a percentage, a technique often used in recording rot diseases of fruits and tubers. The accuracy of such methods depends on the ease with which the symptoms can be recognised and the adequacy of the sampling methods (Moore, 1969). However, for the majority of plant diseases, including those on cereals, more definitive methods are required, especially when the grade or degree of infection needs to be assessed. Wright (1959), used a grading scale in a survey of diseases in wheat crops in Canterbury, describing infection as 'absent, trace, moderate or severe'. However, if different workers are to be able to compare their experiences, a quantitative measurement of the different degrees of infection is essential. For this reason, descriptive and diagramatic keys have been devised.

2.1 Descriptive Keys

These are a description of plants with varying amounts of disease. The first key of this type was produced for assessing late blight of potatoes (Anon., 1947), and it was subsequently modified by Large (1958). Others exist for apple scab (Croxall, Gwynne and Jenkins, 1952), and tomato leaf mould (Beaumont, 1954).

2.2 Diagramatic Keys

This type of key utilizes standard area diagrams. The first example was produced by Cobb (1892), for the assessment of cereal rust, where diagrams showed the pattern of the disease on wheat leaves, when 1, 5, 10, 20, and 50% of the leaf area was occupied by rust postules. Since then, diagrams for a number of diseases have been produced, and manuals containing keys for cereal diseases (Anon., 1968) and cereal, foiage and field crop diseases (James, 1971) have been printed. These keys are based on a percentage scale, which James (1971) considers has advantages in that both lower and upper limits are uniquely defined; the scale is flexible in that it can be conveniently divided and subdivided; and, it could be universally known and accepted.

Both Large (1966) and James (1971) stressed the importance of using growth stage keys for recording the development of the host plant when assessing disease. An example of such a key is the Feekes scale for cereals (Large, 1954), (Fig. 7). Similarly, if the assessment refers to any

particular plant component, for example, leaves, then this fact should be recorded so the meaningful comparisons can be made at a later date. The method of selecting the sample for assessment should also be recorded.

Moore (1969) noted that in compiling and using standard area diagrams, there are two aspects of importance. Firstly, the unaided eye tends to overestimate the amount of infection present, particularly about the 50% level. Also, up to this level, the eye tends to assess the diseased areas, while over this level, it tends to assess the healthy tissue. Secondly, visually distinguishable infection levels are often in logarithmic sequence, and hence a series of levels each double the one below appears to give the most helpful series. Most of the successful and widely used scales have some or all of the levels 1, 5, 10, 25, 50, 75 and 100% infection.

In this work, a diagramatic key (Anon., 1968), with a scale of 1, 5, 10, 25, 50, and 75% infection (Fig. 8) was used to measure the percentage infection of leaves by *Septoria* fungi during the different growth stages of the wheat plant. It was also adopted in an experiment designed to measure the yield loss due to *Septoria*, and the effectiveness of fungicides for control of the disease.

C H A P T E R III

FUNGICIDES

A fungicide can be broadly defined as an agent that kills or inhibits the development of fungal spores or mycelium. The fungicides used on plants may be classified as eradicant, protectant or systemic on the basis of their uptake by, and mobility within, plant tissues. (Wain and Carter, 1972).

3.1 TYPES OF FUNGICIDES

3.11 Eradicant Fungicides

Wain and Carter (1973), define these as fungitoxic chemicals which, when applied at an infection site, are capable of limited penetration leading to the elimination of an established infection.

3.12 Protectant Fungicides

These are substances which protect whole plants or plant parts from fungal infection, by killing or inhibiting the development of spores or mycelium at the site of infection. Protectant fungicides which may be applied to seeds, soil or the plant surface, cannot penetrate into plant tissues in effective amounts. They must therefore, act outside the plant prior to infection by the pathogen. Effective use of protectant fungicides requires application to plants before the arrival of inoculum.

Protectant fungicides for control of foliage diseases have some limitations, as the timing of applications, the incomplete coverage of host surfaces with spray applications (Livingstone, 1953), and the rapid loss of protectant deposits with rain (Rowell, 1964), can all create problems. The difficulties experienced with these factors often make protectant fungicides unsuitable for the control of foliar diseases. However, when protectant fungicides are applied to seed, good control of external seed-borne pathogens can be achieved.

3.13 Systemic Fungicides

A systemic fungicide is any fungitoxic compound capable of being absorbed and freely translocated within the plant (Wain and Carter, 1972). Ideally, a true systemic fungicide is a compound that is taken up by the roots and moves 'in toto' to all parts of the plant where it provides protection against pathogenic fungi (Woodcock, 1968). In practice, it may not be the applied chemical which moves, but a metabolic product, or even conjugates of the metabolite, or of the original compound. Provided that the transformation products retain fungitoxicity and biomobility, without phytotoxicity, the end result is the same.

While disease control was based on the use of protectant fungicides, the development of systemic fungicides proceeded only slowly. It was not until the 1940's that a great deal of work was initiated and aimed at obtaining compounds which were systemic and fungitoxic but not phytotoxic. Systemic

fungicides became of major interest in 1964 with the discovery that the antihelminthic, 'Thiabendazole', was antifungal (Robinson, Phares and Graessle, 1964). As a consequence, many new systemic fungicides have been developed.

3.2 METHODS OF APPLICATION FOR SEPTORIA CONTROL

3.21 Seed Treatment

Evans (1972) defines a seed dressing as a substance applied to seed for the control of pest or disease problems of the species. Before the appearance of systemics, the functions of seed dressings were to destroy any pathogenic organism carried on or with the seed, and to protect the seed during germination from the attack of soil-borne pathogens (Evans, 1968). However, the introduction of systemic fungicides has added two further possibilities - the control of pathogens situated within the seed and previously inaccessible to chemicals (e.g. *S. nodorum*, *Ustilago nuda*), and to control air-borne diseases using the dressing as a reservoir of fungicide for root uptake during the growth of the crop.

The use of seed dressings is the simplest and certainly the most economical method of disease control in present day usage, (Evans, 1968), but the value of the method for controlling *Septoria* diseases is doubtful. Shipton et al. (1971) report that mercurial dusts and dithiocarbamate slurrys have been successfully used in Europe as seed dressings for the

control of *S. nodorum*, while Darpoux et al. (1968) and Ventura et al. (1970) found that the systemics thiabendazole, triamirol and benomyl were active against *S. nodorum* in seedlings. Sanderson (1974) obtained control of *S. tritici* for 6 weeks after seedling emergence through applying benomyl as a seed dressing. However in many countries the importance of seed as an inoculum source for *Septoria* is not clear (Shipton et al., 1971) and so there is doubt as to the value of seed treatments, when other inoculum sources exist (e.g. ascospores from perithecia in stubble).

3.22 Foliar Treatment

Air-borne inoculum can often lead to the rapid development of epidemics. This means that spray or dust treatments of the aerial parts of the crop must be used to eradicate infections, or to protect plants. The aim is to apply a uniform covering of chemical to all susceptible parts of the plant, but the method in which this is attempted will depend on the growth form of the crop.

Where economically possible, a series of treatments designed to cover all potentially infective periods is applied. The efficiency of any treatment will therefore depend on the persistance of the active ingredient between one treatment and the next, and the distribution of the ingredient during the infective periods. Non-systemic products often have to withstand severe weathering as surface deposits, while systemics are generally less dependent on weather because they are able to penetrate host tissues (Evans, 1972).

The distribution of the active product on or in the host plant is an important factor governing efficiency of any spray deposit. With non-systemics, final distribution depends largely on the method of application used, and the redistribution of the deposits as solutions, suspensions and vapours on the surface of the crop (Evans, 1968). Systemics on the other hand, can penetrate tissues and move in the plant, so that their distribution is usually superior to that of non-systemics, although the actual compound that reaches the site of action may not always be the chemical supplied in the formulated product.

Caldwell and Narvaes (1960), Sanderson (1964b), Shipton (1968), Jenkins and Morgan (1969) and Melville and Jemmett (1971) all achieved partial control of *Septoria* diseases by spraying with dithiocarbamates, but most of these were with uneconomical and impractical spray schedules. To date, few systemics have been evaluated for *Septoria* control, most work with wheat being on the selection of systemics for rust and powdery mildew control. Brooks (1972) reported good control of *S. nodorum* with programmes of benomyl sprays, and Hampel and Pommer (1972) found that application of a benzimidazole derivative (BAS 3460F) to wheat at ear emergence increased yields by 7-10%. Shipton et al. (1971) noted that this aspect of disease control has not been investigated sufficiently, especially on the economics involved in foliar spraying for control of the *Septoria* diseases of wheat.

3.3 TIMING OF TREATMENTS

The overall efficiency of any fungicide treatment is eventually dependent on accurately timing the application in a manner dictated by the biology of the pathogen (Evans, 1972). This was especially important with protective fungicides, but with some systemics is less important due to tissue penetration giving improved distribution and weathering characteristics.

The aim with fungicide application is to attack the pathogen at the weakest point in its life cycle (Evans, 1968). This is when the inoculum potential is minimal i.e. the pathogen population is at its lowest, the host least susceptible to the fungus, and the environmental conditions least congenial to infection. This principle can be applied to seed and soil-borne pathogens by treating seed and soil prior to planting, but it is more difficult to apply to airborne pathogens as they tend to be extremely mobile, and re-infection from outside the treated area can occur. With protectant fungicides this leads to the use of routine spray schedules throughout the infective season (Evans, 1972), with the actual interval between successive treatments being dependent on the growth rate of the crop as well as the dosage used.

The internal movement of systemics means theoretically that previously unsprayed tissues may receive toxicant, i.e. a translocation of systemic product from older sprayed organs to newly developed structures. However, there is no evidence to suggest that any of the present systemic fungicides can

move downward and then to the terminal buds of the plant (Evans, 1972). Until such products are available, systemic fungicides will continue to rely on their limited movement within the leaves or stems to which they are applied, and in their powerful eradicant activity (Brooks, 1972).

In many cases, the economic return from disease control does not normally justify more than one spray application, so that timing of spray application is vitally important. However, it would be wrong to standardise spray timing recommendations in terms of time of year or stage of growth of crop, since these will vary from area to area and from year to year. The optimum time to spray is probably at the beginning of the rapid phase of epidemic build-up (Brooks, 1972). The way in which the pathogen affects the plant must also be taken into consideration i.e. whether infection will reduce tiller numbers, or reduce grain weight through flag leaf and head infection. Jones (1974) considered that in Britain, *Septoria nodorum* inflicted most damage on the wheat plant through flag leaf and head infection, and recommended spraying at growth stage 9 to achieve control.

3.4 MODE OF ACTION OF FUNGICIDES

For any compound to be an effective fungicide, its chemical structure must enable it to have access to the site of some biochemical process that is essential to the life of the parasite; must be capable of reacting with some essential

component of the biochemical system; and must show some degree of specificity i.e.an ability to inhibit the parasite without harming the host, due to either a qualitative or quantitative difference in response by host and parasite (Evans, 1968).

Kars Sijpesteijn (1972) distinguished 3 different methods by which fungicides can exert their action on the fungal cells and not on the host cells:

a) inhibition of energy production, by inhibition of respiration or by uncoupling of oxidative phosphorylation. The energy-producing processes are located in part in the cytoplasm, and in part in the mitochondria.

b) interference with bio-synthesis e.g. of protein synthesis, DNA and RNA synthesis and the production of low molecular weight compounds such as amino acids, purines, pyrimidines and vitamins.

c) disruption of cell structures:- fungicides may affect permeability of the cell membrane resulting in leakage of cell contents.

At present, far more is known of the antifungal action of protective fungicides than of systemic fungicides. The exact biochemical mode of action is not yet fully understood for any systemic fungicide.

In field trials against *Septoria* diseases in wheat (Sect. 6.2) 3 fungicides were used, and their modes of action will be discussed. These 3 fungicides were benomyl and thiophanate-methyl, which are both systemic, and mancozeb, which is a protective fungicide.

3.41 Benomyl

Investigations by Bollen and Fuchs (1970), and Edgington et al. (1971) on the fungicidal spectrum of benomyl revealed that this chemical was selective, and inhibited mainly fungi belonging to the Ascomycetes. In aqueous solution, benomyl has been shown to be rapidly hydrolysed to methyl-benzimidazole-2-yl carbamate (MBC) (Clemons and Sisler, 1969), and it is now thought that this stable breakdown product is responsible for the fungitoxicity Sims et al. (1969) were able to detect MBC and not benomyl in cotton plant extracts 4 weeks after treatment, while Peterson and Edgington (1969) found that benomyl completely transformed to MBC in 5 days in bean plants.

Clemons and Sisler (1971) concluded from their studies that the mode of action of MBC was to inhibit DNA synthesis, or some closely related process such as nuclear or cell division. As yet, the exact mechanism has not been determined.

3.42 Thiophanate-methyl

Aelbers (1970), and Matta and Gentile (1971) found that thiophanate-methyl and thiophanate were similar in action to benomyl, and Bollen (1972) found that the antifungal spectrum of the thiophanates closely paralleled that of benomyl.

Selling et al. (1970), and Vonk and Kaars Sijpesteijn (1971) found that thiophanate-methyl in tap-water, buffer or sterile nutrient medium formed MBC. From this it is obvious that benomyl and thiophanate-methyl must have the same mode of action.

3.43 Mancozeb

The ethylene bis-dithiocarbamates are complex chemicals, and it is difficult to study their mode of action (Lukens, 1971). The active form of the mancozeb compound is not certain, but Morehart and Crossan (1965) believe that disulphide is the fungitoxic component.

The dithiocarbamates tend to be fungitoxic through being chelating agents. It is known that maneb disturbs the tricarboxylic acid cycle of the fungus *Neurospora sitophila* through the inhibition of aconitase (Owens, 1960). This is achieved presumably by removing the iron co-factor.

There is some evidence to indicate that chelating agents such as the dithiocarbamates act by attacking thiols of fungal membranes, or by removing metals from the membrane (Lukens, 1971), and this could be the case with mancozeb.

SECTION A 2: EXPERIMENTAL

C H A P T E R IV

THE PATHOGENS

4.1 INTRODUCTION

In New Zealand, reports of *Septoria* diseases of wheat have cited *Septoria tritici* as the causal organism, and no mention has been made of *Septoria nodorum*. However, in most other countries the two are generally associated, i.e. where *S. tritici* is present *S. nodorum* is also usually found (Shipton et al., 1971). Although the presence of *S. nodorum* in New Zealand has been noted, (Anon, 1953; Sanderson, 1972b), it seems that it has never been isolated into pure culture for positive identification. Because of this, work was initiated to confirm the presence of *S. nodorum* in New Zealand.

4.2 ISOLATION

4.21 Introduction

During this study, both *S. tritici* and *S. nodorum* were isolated from two sources obtained from the field, viz. wheat leaves and stubble debris. *S. tritici* was found to be infecting leaves of cv. Aotea in the 1973 wheat trials, and successful isolations were made from this material. However

S. nodorum could not be found at this stage. It was not until the stubble from this trial was examined that the perithecia of both fungi were found. From the ascogenous state, *Leptosphaeria nodorum*, ascospores were obtained, grown in culture, and these produced the pycnidial stage (*S. nodorum*). Subsequently, symptoms similar to those caused by *S. nodorum* were found on volunteer wheat plants during the winter and spring of 1974, and from this material *S. nodorum* was again isolated. Therefore, both pathogens were successfully isolated from the ascogenous state in stubble, and the pycnidial state in living wheat tissue.

4.22 Isolations from the stubble

During the autumn and early winter of 1974, wheat stubble was examined, and this revealed the presence of perithecia embedded in old leaf tissue. These appeared to be those of *Leptosphaeria nodorum*, the ascogenous state of *Septoria nodorum*, and a *Mycosphaerella* sp., the ascogenous state of *S. tritici*. This was confirmed by comparing them with published descriptions (Anon, 1966; Sanderson, 1972a).

Procedure.

An attempt was made to isolate the *L. nodorum* and *Mycosphaerella* sp. that were present, using ascospores after the method described by Sanderson (1972a). A number of glass petri dishes, each containing a glass microscope slide, were, after sterilization, poured with a thin layer

of 1% water agar (Davis Gelatine N.Z. Ltd), so that the slide in each dish was covered with a thin layer of agar. Small pieces of dry leaf tissue containing perithecia were cut and soaked in distilled water for 20 minutes. These were then attached to the inside of the petri dish lid by petroleum jelly so that the perithecia were pointing down towards the water agar. The dishes were then placed in an incubator at 24°C in the dark.

After 12 to 16 hours, the slide was examined under a stereoscopic microscope for the presence of germinating ascospores. Once identified as ascospores typical of either *Leptosphaeria nodorum* or *Mycosphaerella* sp., single spores were transferred onto potato dextrose agar (PDA, 'Oxoid' dehydrated media) by means of a sterilized needle. These single spore cultures were then incubated at 24°C in the dark.

Results.

After incubation at 24°C, all colonies remained white, but there were differences in growth rate and colony morphology. Colonies from the *Leptosphaeria* ascospores filled the entire plate in 25 days. The mycelium in these colonies was grey-white, sparse and flat. After 30 days, small tufts of pure white mycelium were thrown up throughout the colony, but by 40 days, no signs of sporulation were apparent.

The *Mycosphaerella* colonies at 25 days averaged 2.5 cm diam., and were composed of dense white mycelium which was slightly raised in the centre. At 40 days, colony size averaged 2.9 cm diam., but there were no signs of sporulation.

4.23 Inducing sporulation

The failture of the colonies grown from *Mycosphaerella* ascospores to sporulate is surprising, as Sanderson (1972a) readily obtained spores from cultures developed in the same manner. However, Cooke and Jones (1970) found PDA to be a poor medium for sporulation of *S. tritici* and *S. nodorum*. The procedure described by Cooke and Jones (1970) was then used to initiate sporulation by these cultures. These workers used different media under different light regimes, and found that near ultra-violet (NUV) irradiation (3,100 to 4,100 ^{o}A) increased the sporulation of both pathogens. Subcultures of colonies from both the *Mycosphaerella* and *Leptosphaeria* ascospores were developed on malt agar, PDA and oatmeal agar. The malt and PDA were both 'Oxoid' dehydrated preparations made up according to the manufacturer's directions. The oatmeal agar was based on a recipe described in Tuite (1969). To counteract drying of the media under artificial lights, all plates were poured with 40 ml of agar. At first, glass petri dishes were used, but later clear plastic disposable petri dishes were used as these transmit a greater amount of NUV irradiation (Leach, 1967).

To enable the colonies to establish on the media, they were incubated in the dark at 24^{o}C. Colonies from the *Leptosphaeria* ascospores were left for two days, while those from *Mycosphaerella* ascospores, because of their slow growth rate, were left for 10 days. The colonies were then placed in a cabinet and given 12 hours NUV irradiation and 12 hours dark.

The NUV tube was a Philips TL 40w/08, suspended approximately 45 cm above the plates inside an enclosed cabinet maintained at 22 ± 2°C.

Colonies were examined at regular intervals for the presence of spores. Subcultures were made approximately every fortnight, so that the young colonies were always being irradiated.

Results.

For three months, cultures of both types grew only vegetatively, in all treatments. The temperature in the light cabinet was lowered to 20°C and raised to 24°C; the photoperiod was increased to 24 hours, and numerous subcultures were made, but no colony formed spores, either under light or dark incubation.

However, nearly 4 months after the original colony had been grown from *Leptosphaeria* ascospores, examination of a 30 day old colony on oatmeal agar in the dark at 24°C, revealed the presence of dark brown pycnidia under the mycelial mass. Microscopic examination showed that these contained spores of a type strongly resembling those of *S. nodorum*, as described by Anon (1966). These pycnidiospores were 21-29 x 2.5-3 µm, hyaline, three septate, and generally rounded at both ends. Subcultures from this sporulating colony were placed under NUV irradiation, and formed spores after 18 days (Plate 1).

Although the colonies grown from the *Leptosphaeria* ascospores eventually sporulated, those from the *Mycosphaerella* ascospores did not, and the reason for this is not known.

Colony morphology and growth rate were similar to those colonies produced by field isolates of the conidial stage (*S. tritici*), which sporulated either in the dark or when exposed to 12 hours NUV. Thus it can only be assumed that the colonies grown from the *Mycosphaerella* ascospores were those of *S. tritici*.

4.24 Isolation from wheat plants

S. tritici was found infecting leaves of cv. Aotea in the 1973 wheat trials, and also infecting volunteer wheat plants in the stubble of these trials. It was possible to isolate the fungus from both of these sources. However, during 1973 *S. nodorum* could not be found on plants in the wheat trials, and it was not until late winter, early spring of 1974 that symptoms typical of *S. nodorum* were found on the bottom leaves of volunteer wheat plants in the stubble of the 1973 crop. Isolations were made from this material.

During this work, the same procedures were used for both *S. tritici* and *S. nodorum*. All wheat tissue, from which isolations were to be attempted, was first surface sterilized in a solution containing 0.5% available chlorine for five minutes, and then washed twice in sterile water. Once the material had been prepared in this way, a number of different isolation techniques were tried. All isolations and transfers were made under a transfer hood with filtered air.

a) Direct plating

Standard aseptic procedures were used to place 2 cm pieces of diseased tissue on the surface of modified PDA (PDA plus 50 ppm chloramphenicol to prevent bacterial contamination). In general, 3 pieces of tissue were placed in each petri dish, which was then incubated at 24°C in the dark.

b) Keitt single spore method

Using sterile forceps, a small piece of tissue containing pycnidia was transferred to a dry, sterile petri dish. Four drops of sterile water were placed onto the tissue which was then macerated. Part of the resulting spore suspension was streaked into petri dishes of modified PDA. Plates were then incubated at 24°C in the dark and examined after 72 hours. Well isolated spores were selected and their positions indicated with a marker pen. Small agar segments containing these spores were then transferred by needle to a second plate of modified PDA, which was then incubated at 24°C in the dark.

c) Plating spores exuded from pycnidia

Diseased tissue containing pycnidia was washed twice in sterile water and examined under a dissecting binocular microscope. Using flamed needles, pressure was applied equally on both sides of a pycnidium, causing spores to exude and form a spore horn over the ostiole. This spore mass was then carefully transferred by a needle to a drop of sterilized distilled water in a sterile petri dish. The drop was stirred to disperse the spores. From this suspension, loopsful were streaked across petri dishes of modified PDA. The plates were then incubated at 24°C in the dark.

Results.

Isolates of both *S. tritici* and *S. nodorum* were obtained by all three methods. However, the technique of direct plating leaf tissue onto modified PDA did not prove as successful as the latter two methods. After 4-6 days, white mycelial growth was often observed, but bacterial contamination generally suppressed fungal growth. By this method, *S. tritici* was isolated once out of fifteen attempts and *S. nodorum* twice out of ten attempts.

Some bacterial contamination was also experienced with both spore suspension methods, but by subculturing single spore colonies at an early age, pure cultures were readily obtained.

4.3 PROOF OF PATHOGENICITY

4.31 Introduction

Before a particular organism can be claimed as being responsible for a previously defined diseased condition, 'Koch's Postulates' for proof of pathogenicity must be fulfilled.

The pathogenicity of *S. tritici* in New Zealand was proved by Wenham (1949) for both the black and white cultural races which were isolated from wheat tissue. However, Wenham did not isolate *S. nodorum*. Although its presence in New Zealand wheat crops has been recorded (Anon, 1953; Sanderson, 1972b),

its identity has not been positively proven. After the successful isolation of *S. nodorum* both from stubble and wheat plants (4.2), experiments to prove its pathogenicity, as well as those of the *S. tritici* isolates, were initiated. Isolates from four sources were tested:-

a) *S. nodorum* - ex *L. nodorum* ascospores from perithecia formed on stubble.

b) *S. nodorum* - ex pycnidia from volunteer wheat

c) *S. tritici* - ex pycnidia from volunteer wheat

d) *S. tritici* - ex *Mycosphaerella* sp. ascospores from perithecia formed on stubble*

* Colonies grown from this source did not sporulate in culture, and only the mycelial inoculation method could be used.

4.32 Inoculation

On PDA and oatmeal agar, both pathogens produced pycnidiospores after 20-30 days of growth. Most inoculation techniques involved the application of spore suspensions prepared from actively growing colonies. A colony was removed from the agar and shaken vigorously in sterile distilled water, so that mycelial fragments and pycnidiospores were in suspension. A haemocytometer was used to determine the concentration of spores, which was adjusted to 1×10^6 spores/ml. Two drops of 'Tween 20' surfactant were added to each 100 ml of suspension.

In all inoculations for proof of pathogenicity, the host plants were the susceptible wheat cv. Aotea (Sanderson, 1964b). The seed was sown in loam soil in 10 cm pots, four seeds per pot. These were placed in a glasshouse, and left for just over 3 weeks, at which stage the plants were approximately 15 cm high and had 3 leaves. Once the plants were at this growth stage, inoculations were made using two methods; whole plants and detached leaf culture (defined as 'the maintenance of leaves on a liquid or agar medium, usually for several days or weeks' (Tuite, 1969).

a) Whole plants

A number of different methods were used to apply the inoculum.

i) Spore suspension spray.

Wheat plants were spray inoculated using a hand atomizer (No. 15-De Vilbiss Co., U.S.A.), until runoff.

ii) 'Painting' technique.

A sterile paint brush was used to place the spore suspension on the adaxial surface of the leaf.

iii) Placement of an agar culture of the pathogen on the adaxial surface of the leaf.

With the spray and 'painting' techniques the plants were placed immediately after inoculation into a humidity chamber maintained at approximately 100% R.H. After 48 hours the plants were removed to a glasshouse at 20°C and 80% R.H.

When mycelial inoculum was placed on the leaf, a plastic bag was placed over the pot containing the plants. The plants were left in these high humidity conditions for 48 hours, and then transferred to the glasshouse.

Twelve plants (three pots) were inoculated by each method, for both *S. tritici* and *S. nodorum*. With each method eight plants (two pots) were similarly treated, but sterile water or agar medium was substituted for the inoculum.

Results.

Successful inoculations using isolates a, (*S. nodorum* ex *L. nodorum* ascospores), b, (*S. nodorum* ex pycnidia), and c, (*S. tritici* ex pycnidia) were made to Aotea wheat plants by both the spore suspension and 'painting' techniques. The disease was more severe when the 'painting' technique was used (due presumably to a high spore concentration on the leaves, and better distribution obtained by this method). When mycelium only, from all four isolates was applied to the leaves, in some cases, after two weeks there was a slight chlorosis of the leaf around the site of application, but no lesions developed.

Infection by *S. nodorum* was evident 10 days after inoculation, when necrotic water-soaked lesions appeared. By 16 days, discrete necrotic lesions had developed. These were brown in the centre, with a yellow tinge around the outer margins. At 20 days in some cases, the lesions had coalesced across the leaf, but in general they tended to remain discrete.

At this stage there was heavy chlorosis around the outer margins of the lesions. By 28 days, many of the infected leaves had necrotic lesions over 80% of their leaf area, and any remaining tissue was completely chlorotic. However, even after 34 days, no pycnidia had formed. (Plate 2).

Infection by *S. tritici* was slower than that by *S. nodorum*. Symptoms began to appear between 16-20 days after inoculation, as small chlorotic dots on the green tissue. These elongated, often coalescing with neighbouring spots, and extensive areas of the leaf became chlorotic. At 25 days a number of lesions contained small golden-brown pycnidia, and by 34 days, these were mature and black. (Plate 3).

With the methods of inoculation used for *S. nodorum* lesions developed, but no pycnidia formed. This also happened in several instances with *S. tritici*. In experiments with *S. tritici* Wenham (1949) found that in some cases pycnidia did not form, and returning such plants to an atmosphere of high humidity initiated pycnidial production. In my experiments, if pycnidia had not formed after 30 days, the inoculated plants were placed in high humidity for 48 hours. Pycnidia of *S. tritici* then were formed a week after this treatment. However, with plants inoculated with *S. nodorum*, pycnidia still were not produced. Pieces of leaf with lesions of *S. nodorum* were then removed from the plant and floated on 10 ml of 50 ppm benzimidazole solution in a petri dish. The dish was placed under NUV light with a 12 hour photoperiod, and pycnidia of *S. nodorum* formed after 3 days.

In all inoculation techniques, the check plants remained healthy, with no evidence of lesions or pycnidia.

b) Detached leaf culture.

Detached leaf culture has been used for testing resistance to *S. tritici* (Sewell and Caldwell, 1960; Baker, 1970), and *S. nodorum* (Baker, 1970). Benzimidazole was used as a medium to maintain the detached leaves, because of its ability to maintain the protein and chlorophyll content of the leaf (Samborski, Forsyth and Person, 1958). However, water and kinetin have also been used (Tuite, 1969).

Two techniques were used to determine pathogenicity of both *S. tritici* and *S. nodorum* by this method, using benzimidazole at a concentration of 50 ppm.

i) The first method was that described by Baker (1970). Excised leaf portions (4 cm in length) from 14 day old Aotea wheat plants were first stroked lightly between finger and thumb to remove the bloom. They were then painted on their adaxial surfaces with the spore suspensions. The leaf portions were placed adaxial side uppermost in petri dishes containing 5 ml of 50 ppm benzimidazole, two leaves from each plant in the same dish. Two sets of four dishes of each treatment were established, and a further set of dishes contained leaves treated with water only. One set of each fungus and water treatment was

left on a bench at room temperature, and examined after 10-15 days, while the other set was placed in a growth cabinet at 20 $\pm$ 1°C, with a 16 hour photoperiod.

ii) The second method was a modification suggested by Jones (1974). The excised portions were the distal 4 cm of the wheat leaf. Two sterilised glass rods were placed in each petri dish, and the cut end of the leaf was placed under one rod, and in the benzimidazole solution. The uncut end of the leaf was over the top of the second rod, and so out of the benzimidazole solution. The spore suspension was then painted onto that portion of the leaf which was out of the solution. This was to try and counter any possible effects of the benzimidazole on the germination of spores of the pathogen.

As with the floating method, two sets of four dishes of each treatment were established, one set at room temperature on a bench, and the other at 20 $\pm$ 1°C in a growth cabinet. Water was applied to sets of leaves which acted as a control.

Results.

With both fungi, symptoms developed more quickly with the detached leaf than the whole plant technique. This may be because the detached leaf was less able to withstand

attack by the pathogen, for as soon as they are cut, the leaves begin to senesce, and the benzimidazole can only slow down the rate of senescence, not prevent it.

With *S. nodorum*, necrotic lesions developed after 6 days, and by 12 days these were 5-6 mm in length and dark-brown, with yellow outer margins. At 16 days, most of the leaf tissue outside the lesions was chlorotic. As with the whole plant technique, no pycnidia had developed after 30 days, but when these detached leaves with lesions were placed under NUV light, pycnidia developed 3 days later. (Plate 4).

With *S. tritici*, 1-2 mm chlorotic spots appeared after 12 days and indicated that infection had occurred. After 21 days, well-defined lesions were present, with pycnidia also present. No lesions appeared where the leaf tissue was actually submerged in benzimidazole. There were no differences between the two techniques or growth regimes used, in either symptom expression or pycnidial formation. In all cases, control leaves remained green throughout the duration of the experiment.

4.33 Re-isolation of the pathogen

To fulfil all of Koch's postulates, the fungus, once seen to be capable of infecting the plant, must be re-isolated from the infected tissue. It must then be compared with the original culture, to determine that the fungus causing the disease is the same as the one which was inoculated into the plant.

Successful isolations were made from pycnidia from both the whole plant and detached leaf techniques, but isolation was easier from the former. Two of the previous methods were used; either a pycnidium was removed from the leaf tissue with the aid of a flamed scalpel, and macerated in a drop of sterile water, or a mass of spores collected from the ostiole of a pycnidium and transferred to a drop of sterile water. The resulting spore suspensions were streaked onto modified PDA with a flamed loop, and the plates incubated at 24°C in the dark.

As in the original isolations, growth of *S. nodorum* was discernible after 2 days, and *S. tritici* after 4 days, at which time minute colonies could be distinguished. Single spore colonies of *S. nodorum* and *S. tritici* were obtained from the leaves inoculated by the two techniques. However, with the detached leaf method, a number of attempts were required before successful isolations were achieved as the high numbers of bacteria on the surface of the senescing leaf meant that it was difficult to obtain pure colonies of the fungus. Before extracting a pycnidium, the leaf was washed in a solution containing 0.5% available chlorine for 3 minutes to remove a large number of contaminants, and this allowed single spore colonies to be isolated from the path of the streak.

4.34 Comparison with the original cultures

With both *S. nodorum* and *S. tritici*, no differences could be detected between the re-isolated fungal colonies, and the original isolates from which the inoculum was prepared. On the same media, growth rate, surface topography, edge and colour of the colonies were identical. Microscopic examination revealed the same spore and mycelial structure for each pathogen respectively. It was established beyond doubt that the fungi isolated from lesions on artificially inoculated plants were the same as those from which the inoculum was originally produced. Proof of pathogenicity had been established for both fungi, and so *S. tritici* and *S. nodorum* are pathogens of wheat.

4.4 CULTURAL STUDIES

To study cultural features, it was necessary to obtain monosporous isolates of *S. nodorum* and *S. tritici*, and grow them under controlled environmental conditions. Spore suspensions, from both naturally and artificially infected leaves were streaked onto PDA, using the Keitt platinum loop technique. These plates were then incubated at 24^{o}C in the dark.

4.41 *Septoria nodorum*

Minute colonies appeared after 2 days, were pure white, and averaged 0.4 mm in diameter. Individual colonies were

then removed to different culture media (PDA, malt or oatmeal agar) and, after 2 days at 24°C in the dark, placed under two light regimes (12 hr NUV/12 hr dark, and darkness), after the method of Cooke and Jones (1970). Six replicates (i.e. petri dishes) of each treatment were established.

Results.

The effects of NUV irradiation and agar medium on colony morphology are shown in Plates 5-7, and in Table 1. All colonies were originally white, but after 10 days growth had begun to change to greys and browns, depending on the medium. At 20 days, there was a variation in colour, depending on medium and light treatment, but there was a tendency towards pink in the mycelium. By this stage, mycelial growth had covered the whole plate, using PDA and oatmeal agar.

With colonies on PDA and malt agar, sporulation did not occur until the mycelium covered the whole plate (at least 20 days). However, colonies on oatmeal agar under NUV light sporulated between 15-20 days. Although Cooke and Jones (1970) found spore cirrhi in colonies on PDA and oatmeal agar, they did not occur in this case. Pycnidia appeared around the edge of the colony or underlying the outer mycelium, and were dark brown to black, 135-195 µm in diameter, with a wall four or five cells thick. Apart from the colour, there was no difference between these and pycnidia found in the field, or on artificially inoculated wheat. Pycnidiospores were also similar to those obtained from the field. They were hyaline and generally straight, either two or three septate, with rounded ends and measured 21-29 x 2.3-3.1 µm.

TABLE 1: Colony characteristics of *Septoria nodorum* grown at 24°C on three media under two light conditions.

Age of Colony (days)	Media	Light Regime: Continual dark			Light Regime: 12 hrs NUV/12 hrs dark		
		Diam. (mm)	Colour	Edge	Diam. (mm)	Colour	Edge
6	Oat	27	White	Entire	31	White	Entire
	PDA	24	"	"	25	"	"
	Malt	19	"	"	17	"	"
9	Oat	40	White/brown	Entire	47	Brown/white	Entire
	PDA	43	White/grey	"	42	White/grey	"
	Malt	30	White/grey	"	24	White/grey	"
12	Oat	52	White/brown	Irregular	60	Brown/white	Irregular
	PDA	61	White/grey	"	59	White/grey	"
	Malt	40	White/grey	Entire	39	White/pink	Entire
19	Oat	Whole Plate	White/pink	Irregular	Whole Plate	Brown/white	Irregular
	PDA		White/pink	"		White/pink	"
	Malt	53	White/pink	Entire	51	White/pink	Entire
26	Oat	Whole Plate	White/pink	Irregular	Whole Plate	Brown/white	Irregular
	PDA		White/pink	"		White/pink	"
	Malt	54	White/pink	Entire	57	White/pink	Entire

S. nodorum demonstrated photosporogenesis in that more pycnidia were produced under NUV irradiation than under dark incubation. Production of pycnidia on malt agar was poor under either light regime. Sporulation appeared to be more profuse on oatmeal agar under NUV light.

Scharen and Krupinsky (1970) found that single spore isolations of *S. nodorum* did not produce stable cultures of the fungus. They found them variable to the extent that cultures could be selected ranging from those with large amounts of mycelium and few or no pycnidia, to cultures with sparse mycelium and large amounts of pycnidia. By selecting for many pycnidia, and a prostrate form of mycelial growth, a 'stable culture' was eventually selected and a number of sub-cultures had identical cultural characteristics.

All cultures of *S. nodorum* isolated in this study were similar on all media used, with, in general a prostrate form of mycelial growth, and with large numbers of pycnidia. However, after two sub-cultures, the numbers of pycnidia produced diminished rapidly, and by the third or fourth transfer, none were produced. The mycelial growth did not change markedly, but it was not as prostrate as that of the original cultures.

A study of the variability and stability of isolates of *S. nodorum* in New Zealand, similar to the work of Scharen and Krupinsky (1970), would provide some important information about this fungus, especially with regard to variability in pathogenicity and its effects on host resistance.

4.42 *Septoria tritici*

Wenham (1949), during cultural studies of *S. tritici*, isolated two culturally distinct races from wheat tissue, and labelled these as 'black' and 'white' races. The black race was genetically stable for it 'was a natural entity co-existing under field conditions with the more common white form'.

During spring of 1973 and autumn of 1974, spore suspensions were prepared from pycnidia from naturally infected plants, and plated onto 2% malt agar. After 4 days of incubation at 24°C, minute colonies began to appear, and it was at once evident that there were two types, white and black. After 7 days growth on malt agar, the white colonies were 2 mm in diameter, and were entire and flat in appearance. At 12 days, fluffy white mycelium was growing in all directions, and the centres of the colonies were raised 2-3 mm. After 30 days, the colony was 24 mm in diameter, convoluted and pink with white around the margin (Plate 8). No signs of spore formation were evident on malt agar, but on PDA, brown to black pycnidia were formed after 35 days, and these and the spores they contained were similar in every way to those found on naturally infected leaf tissue (Plate 9).

In contrast, the black cultural race appeared after 4 days incubation on malt agar as small grey-white colonies. After 7 days these were 1.5 mm in diameter, grey to green black in colour, and the colony edge was entire. At 10 days the colony had assumed a volcano-like appearance, in that

growth appeared to be vertical rather than horizontal. These colonies were black-green with grey tops to the peaks in growth. There was no loose fluffy mycelium, and all colonies were extremely compact. Colony diameter was no more than 15 mm (Plate 10).

A microscopic examination of these colonies at any stage of growth showed that although pycnidia could not be found, there was underneath the outer thick mass of mycelium, hundreds of spores, most of which strongly resembled those of *S. tritici*. However, also present were short spores which Jones and Lee (1974) described as secondary conidia. These appeared to have formed through budding of germinating pycnidiospores (Plate 11). This process has also been reported in *Septoria linicola* (Sackston, 1970).

After the black race had been isolated, a series of single spores were transferred to malt agar, to see if any changes in colony characteristics occurred during this time, and also to see if the black race would revert to the white race. Six successive generations of the original black isolate were treated in this way, but each colony produced was similar to the original isolate. The same procedure was applied to the white race but again there was no difference between the original and final cultures.

Spore suspensions (1×10^6 spores/ml) of each race were then sprayed until runoff, onto 4 cm leaf portions of Aotea wheat floating on 50 ppm benzimidazole. Symptoms, similar to those caused by *S. tritici* were present after 25 days on both

sets of leaves, and by 35 days, pycnidia of *S. tritici* appeared. For both the black and white races these pycnidia were the same size (85-150 µm) and were similar morphologically. Pycnidiospores were also identical (45-70 x 1.5-2 µm). However, spore suspensions from these pycnidia, when transferred to malt agar, produced pure cultures of the black and white races respectively. Thus it appears that the black cultural form is not another species of *Septoria*, but is a genetically stable form of *S. tritici*.

Apart from Wenham (1949), there have been no other reports of cultural races of *S. tritici*. Further investigations of these two races, inoculated into a range of host cultivars may prove the existance of physiologic races in *S. tritici*.

4.5 FACTORS AFFECTING SPORULATION OF *S. NODORUM* ON LEAF TISSUE.

4.51 Introduction

Although *Leptosphaeria nodorum* was found on wheat stubble during autumn of 1974, *Septoria nodorum* could not initially be found on volunteer wheat plants which grew in the stubble (see Section 4.53). There was abundant *S. tritici* infection (both symptoms and signs) on these plants, and it was difficult to understand why one fungus should be found and not the other, especially when ascospores of both *L. nodorum*

and *Mycosphaerella* sp. had been liberated in the vicinity (see Section 5.3). At the beginning of June, some healthy volunteer plants (G.S.6) were brought into the laboratory, and leaf portions from these plants were floated adaxial side uppermost on 50 ppm benzimidazole solutions in petri dishes on a laboratory bench. Two weeks later, these leaves were beginning to turn yellow, and numerous small golden-brown pycnidia were present in the leaf tissue. These pycnidia contained spores which strongly resembled those of *S. nodorum* in size and shape, and were subsequently proved to be of that species. This observation suggested that infection of these volunteer wheat plants by *S. nodorum* had occurred, but that conditions did not allow symptom expression nor pycnidia formation. The following experiment investigated the factors that might be involved.

4.52 Procedure

Factors that could affect the appearance of pycnidia of *S. nodorum* on leaf tissue from the field are light, temperature, moisture, and possibly senescence of the leaf. A preliminary experiment investigated the importance of darkness. Leaf 2 of volunteer wheat plants showing neither symptoms nor pycnidia was used. Five centimetre portions of this leaf were cut, placed in petri dishes and sets of dishes given the following treatments:

TABLE 2: Treatments in preliminary experiment.

Light regime	Temperature (^{o}C)	Moisture regime		
		(a) dry	(b) benzimidazole	(c) water
Dark	8^{o}			
"	10^{o}	"	"	"
"	18^{o}	"	"	"
"	24^{o}	"	"	"

After 10 days, the leaves were examined and no pycnidia had formed. Therefore light seemed to be a requirement for pycnidial formation.

A factorial experiment was then established with the combination of factors as shown in Table 3. Each treatment was replicated five times. After 10 days, leaves were removed from the dishes and examined under a binocular microscope for the presence of pycnidia of *S. nodorum*.

Results.

Table 3 shows that in addition to light, temperature, moisture and senescence of the leaf tissue appear to be important factors. The greatest number of pycnidia were produced when the temperature was in the 18-24^{o}C range, and when senescence of the leaf was nearly complete. Where the leaves were floated on benzimidazole, which delayed senescence of the leaves, fewer pycnidia were produced. The effect of NUV light was to increase pycnidial production, and this result corresponds with that of Cooke and Jones (1970), who found that *S. nodorum* exhibited photosporogenesis.

Pirson (1960) found that more pycnidia were produced by *S. nodorum* on older tissues than young leaves. In this experiment the same results were obtained, although the 'young' and 'old' tissue was artificially produced, i.e. the tissue floating on water was more advanced in its senescence than that floating on the benzimidazole.

TABLE 3: Number of pycnidia of *S. nodorum* after 10 days incubation under different climatic conditions.

Light Regime	Temp. (°C)	Moisture	% senescence of leaf (avg. 10 leaves)	No. pycnidia per leaf (avg. 10 leaves)
Continuous Fluorescent Light	5°	Dry	80	0
		H_2O	5	0
		Benzimidazole	1	0
	10°	Dry	100	0
		H_2O	50	0
		Benzimidazole	5	0
	18°	Dry	100	0
		H_2O	100	15
		Benzimidazole	75	7
	24°	Dry	100	0
		H_2O	100	25
		Benzimidazole	80	23
12 hrs NUV/ 12 hrs dark	22°	Dry	100	0
		H_2O	100	47
		Benzimidazole	41	23

4.53 Appearance of *S. nodorum* symptoms in the field

Through autumn and winter of 1974, volunteer Aotea wheat plants growing in the stubble of the 1973 crop showed no symptoms of *S. nodorum* infection. However, these same plants under controlled conditions in the laboratory produced pycnidia of *S. nodorum* (Section 4.52).

In the early spring, small brown lesions began appearing on the lower leaves of these volunteer wheat plants, and these developed into symptoms typical of *S. nodorum* i.e. discrete brown necrotic lesions with associated areas of chlorotic tissue. No pycnidia formed in the field, but did so when lesioned leaves were floated on benzimidazole and placed under NUV light in the laboratory. From these pycnidia *S. nodorum* was isolated.

In the field, *S. nodorum* lesions appeared only on the bottom 3 leaves of the wheat plants, and were not found at all on any leaves higher up the plant. In contrast, lesions of *S. tritici* could be found on any leaf of the plant. Portions of leaf 2 and leaf 3 (numbering from the flag leaf down) of the volunteer plants were then floated on 50 ppm benzimidazole solution and placed under NUV light. Pycnidia typical of *S. nodorum* appeared on 21 out of the 25 leaves tested within 4 days. Thus leaves 2 and 3 were infected but symptoms had not developed. Conditions necessary to allow symptom expression by *S. nodorum* in the field in New Zealand are unknown, although age of leaf certainly seems important. Further work is needed for these conditions to be determined.

4.54 Discussion

Climatic conditions during autumn and early winter must have allowed infection of volunteer wheat plants by ascospores of *L. nodorum*, or pycnidiospores of *S. nodorum* to occur. Conditions required for germination of *L. nodorum* ascospores are not known, but for *S. nodorum*, temperatures between 10-28°C and a high humidity for at least 24 hours are essential (Shipton et al., 1971). Again, there is little information as to conditions necessary for symptom expression by *S. nodorum*, but Weber (1922) noted that time for appearance of lesions is dependent on conditions, especially temperature. Thomas (1962) and Shearer (1967) showed that symptom expression was directly related to the length of the humid period after inoculation, in that the longer the humid period, the greater the amount of symptom expression. The humidity factor is also important for appearance of pycnidia, as Holmes and Colhoun (1971) demonstrated that with artificial inoculation of young plants, high humidity produced greater numbers of pycnidia.

Further work is needed to determine why symptoms and signs of *S. nodorum* have been seen so infrequently in wheat crops in New Zealand. This work has indicated that temperature and age of tissue could be critical factors, and it seems likely that humidity is also very important. It is possible that hot dry summers experienced in Canterbury do not allow either symptoms or pycnidia of *S. nodorum* to regularly develop.

C H A P T E R V

EPIDEMIOLOGY STUDIES

5.1 INTRODUCTION

In the period between harvesting and resowing of wheat crops (February to May), plant pathogenic fungi must survive and eventually produce primary inoculum to infect the next crop. The importance of wheat stubble as a source of primary inoculum has been clearly demonstrated for both *S. tritici* (Weber, 1922; Wenham, 1959), and *S. nodorum* (Weber, 1922; Von Wechmar, 1966). Infected volunteer wheat plants could also serve as sources of inoculum of *S. tritici* (Wenham, 1959), while seed-borne inoculum of *S. nodorum* can be important (Shipton et al., 1971). However, pycnidiospores are splash dispersed (Scharen, 1966) and there has been no evidence to suggest that they are windborne (Shipton et al., 1971). Hence it is difficult to see how infection is initiated at a site some distance from any possible inoculum source.

In New Zealand the ascogenous states of both *Septoria* spp. have been found on wheat stubble and identified as *Leptosphaeria nodorum* and *Mycosphaerella* sp. (Sanderson, 1972a). However, little is known about their role in the epidemiology of the *Septoria* diseases. Shipton et al. (1971), after reviewing the evidence, considered that as the perithecial stage was rare it played a minor role in the disease cycle of *S. nodorum*. The perithecial stage of

S. tritici, a *Mycosphaerella* sp., has so far been reported only from New Zealand and little is known about its role in the disease cycle.

5.2 STUBBLE EXAMINATION

During 1974, Aotea wheat stubble left in the field after harvest in January was examined at regular intervals to determine whether the perfect states of the *Septoria* fungi were present and of importance in the epidemiology of the diseases.

5.21 Incidence of fruiting bodies

The stubble in the field after harvest was approximately 40 cm high. Most stems retained the bottom 4 leaves. Every fortnight over the next 6 months, a random selection of 25 stems were removed from the field, and the leaves of these examined for the presence of fruiting bodies of *Septoria* spp. Each of the bottom 4 leaves was removed from the stem of the plant and soaked overnight in clear lactophenol. This wet the dry leaf tissue, removed any debris, and cleared the leaves so as to show any fungal fruiting bodies either in or on the leaves. A piece of double sided cellotape was placed at each end of a microscope slide, and the leaf placed down across the slide so that it was kept rigid and could be positioned under a microscope. The leaf was then examined along an area of 60 mm of leaf blade, under a stereo-microscope (x40) and the number of fruiting bodies counted.

Results.

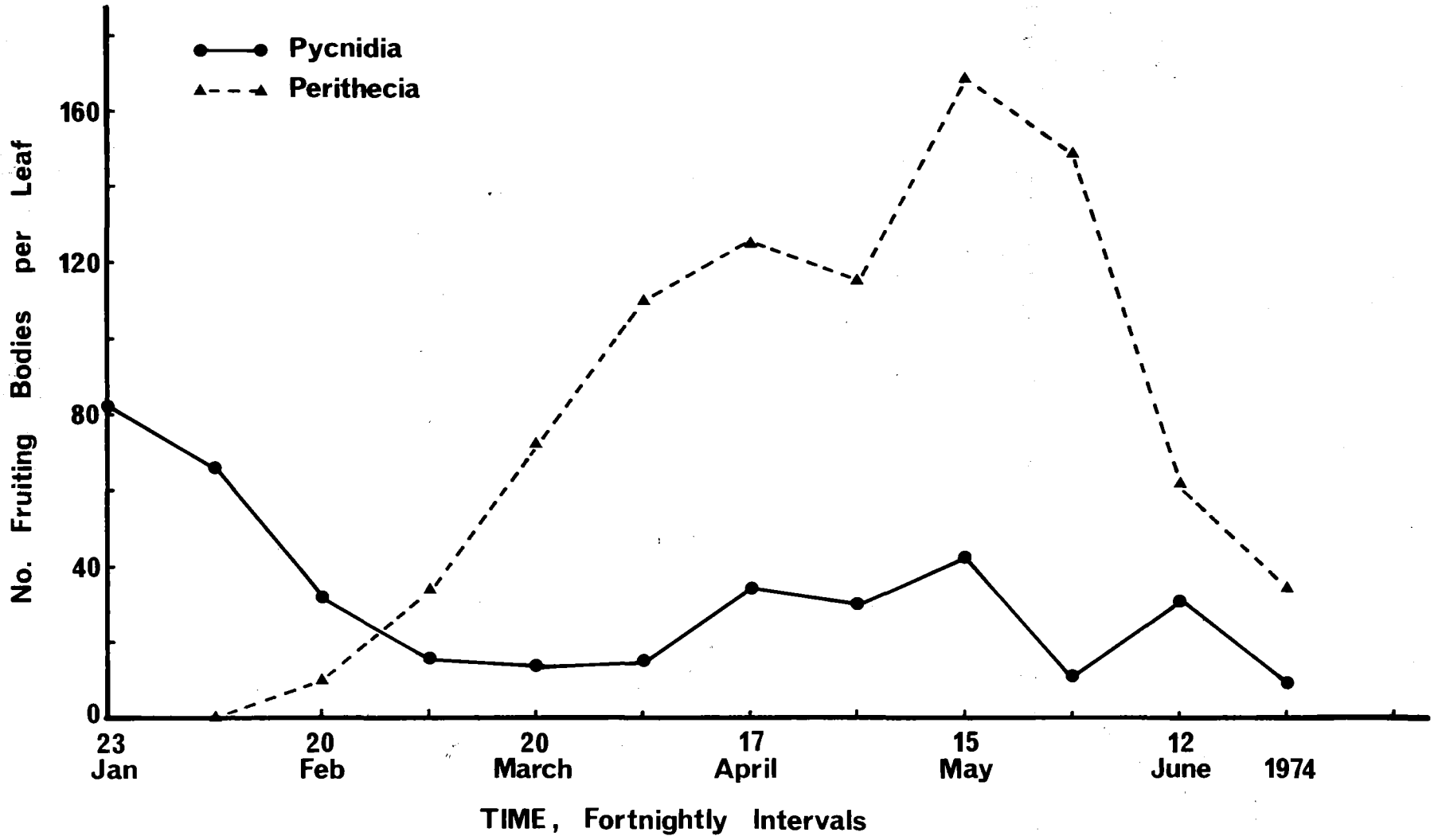
a) Pycnidia

When basal leaf tissue was examined in mid-January, large numbers of pycnidia were present on all leaves. These pycnidia were immersed in the leaf tissue, generally globose, and ranged in colour from a golden-brown to black, and in size from 80-145 µm diam. They were often aggregated into clumps or arranged longitudinally between the veins.

Individual pycnidia were cut from the leaf with a scalpel, mounted in lactophenol cotton blue, and examined under the microscope. This showed that all were pycnidia of *S. tritici*, and contained typical spores. At no stage were pycnidia or spores of *S. nodorum* found.

By mid-February, large numbers of pycnidia were still present in the leaves, but the majority of these proved to be empty. The empty pycnidia were relatively easy to distinguish, as they were flattened and the enlarged ostiole, through which the spores had emerged, was distinct. However, pycnidia at all stages of development were still present, and were found in the stubble through until the end of June, when sampling ended (Fig. 1). It was observed that during April, May and June, there was an increase in the numbers of pycnidia of *S. tritici* (Fig. 1). Scharen (1964; 1966) demonstrated that with *S. nodorum*, additional pycnidia were produced following a period of rain, and that during the drying period there was a replenishment of spores in the older fruiting bodies. This has not been demonstrated before with

FIG. 1 Numbers of pycnidia and perithecia of *Septoria* sp. found on the second leaf from the base of the plant in wheat stubble during January to June, 1974.



S. tritici (Shipton et al., 1971). During April, May and June of 1974 there were heavy showers of rain, and it is possible that the increases in numbers of pycnidia found may have resulted from this rainfall. However, this was not conclusively determined.

b) Perithecia

By the end of February, perithecia began to appear on the lower leaves of the stubble. Under the microscope these were seen to be of two types, subsequently identified as being perithecia of *L. nodorum* and *Mycosphaerella* sp.

Perithecia of *L. nodorum* were immersed in the leaf tissue, globose and dark brown to black in colour, and, when mature, ranged in size from 120-180 µm diam. The perithecial wall was a single layer of 3-4 flattened cells. Asci were club-shaped, bitunicate and 8-spored, 56-78 x 9-12 µm. Filamentous pseudoparaphyses were present. Ascospores were 3 septate, fusoid and hyaline to pale yellow in colour constricted at the septa, and the penultimate cell was swollen. Spore size was 19-23 x 4 µm (Plate 12).

Perithecia of the *Mycosphaerella* sp. were similar to those described by Sanderson (1972a), being globose, immersed in the dead tissue and, when mature, dark brown and 75-95 µm diam. The cell walls were two layered, with the outer one being thickened and pigmented. Asci were bitunicate, obpyriform, 35-40 x 11-14 µm, with the 8 spores usually irregularly arranged. There were no pseudoparaphyses present. Ascospores were 2 celled, elliptical and hyaline, measuring 10-15 x 2.5-3 µm.

When the perithecia were young, it was difficult to distinguish between those of *L. nodorum* and those of the *Mycosphaerella* sp. However, when mature, those of *L. nodorum* tended to be larger and darker than those of the *Mycosphaerella* sp. As it was difficult to distinguish between the perithecia, only total numbers of perithecia were recorded when counts were made.

Low numbers of perithecia were present at the end of February (Fig. 1), but by the end of March there was a marked increase in their numbers. A peak was reached in mid-May, but by mid-June the number of perithecia still containing ascospores had fallen to a low level. Examination of individual perithecia over this time showed that in March, more *L. nodorum* perithecia were mature than those of the *Mycosphaerella* sp., and this observation has been confirmed by the results from spore trapping over this period (Section 5.3).

5.22 Viability of spores

a) Pycnidiospores

Every month from January to June, spores from pycnidia of *S. tritici* were tested for their viability. Pycnidia were excised from stubble tissue collected in the field and soaked in water. These pycnidia were than placed in a drop of sterile water in a sterile petri dish, macerated to liberate the spores, and a drop of the suspension streaked onto 2% water agar. After 2 days incubation at 24°C in the

dark, the petri dish was examined under a microscope and the number of spores that had germinated was recorded.

Results.

Table 4 shows that there was little difference in percent germination from January until April, but from May there was a decline, until in July, viability was very low. However, conditions at 24°C where this germination was measured are not comparable with climatic conditions in the field during autumn and winter. Weber (1922) noted that a temperature of 22-24°C favoured germination of *S. tritici*, but temperatures in the field over the autumn and winter were certainly lower than this. To check on the viability of the spores at lower temperatures, spore suspensions were obtained as before, steaked onto 2% water agar, and incubated at 10°C for 2 days. Table 4 shows that at these lower temperatures viability also declined over the period April to July.

These results correspond with those of Wenham (1949), who found that *S. tritici* pycnidiospores were viable for 5 months (from February to June), but viability then declined rapidly. Thus viable pycnidiospores of *S. tritici* would be available to infect autumn sown wheat plants as soon as they emerge, provided that this inoculum reached the plants successfully.

b) Ascospores

The viability of ascospores of *L. nodorum* and *Mycosphaerella* sp. was determined during the period April to July 1974. Small pieces of leaf blade containing

several ascospores were cut and attached to the lid of a petri dish (with petroleum jelly) so that ascospores could be liberated onto 2% water agar. After 48 hours incubation at either 24°C or 10°C, the surface of the agar was examined, and the percentage of germinating ascospores determined.

Results.

Table 5 shows that at 24°C, viability was high through April and May, but by July was declining markedly. At 10°C, viability was lower than that at the higher temperature, but this may be due to slower germination at the lower temperature. However, over the months April to June, when wind-borne ascospore could come into contact with autumn-sown wheat plants, viability was such that infection could well take place, even at temperatures of 8-10°C.

5.23 Effect of age of tissue on perithecial appearance

During the months of February and March, when perithecia were beginning to appear on the stubble, the age of the leaves which first showed perithecia were determined. In each case, perithecia appeared first on the bottom leaf of the plant (Fig. 2). Although this suggests that age of tissue is the determining factor for appearance of perithecia, this cannot be inferred unequivocably. However, the results in Section 4.5, and those of Pirson (1960), that more pycnidia of *S. nodorum* were found on older than younger tissue, tend to support this view.

TABLE 4: Germination, after 2 days on 2% water agar, of *S. tritici* pycnidiospores collected from pycnidia on wheat stubble during the months January to July.

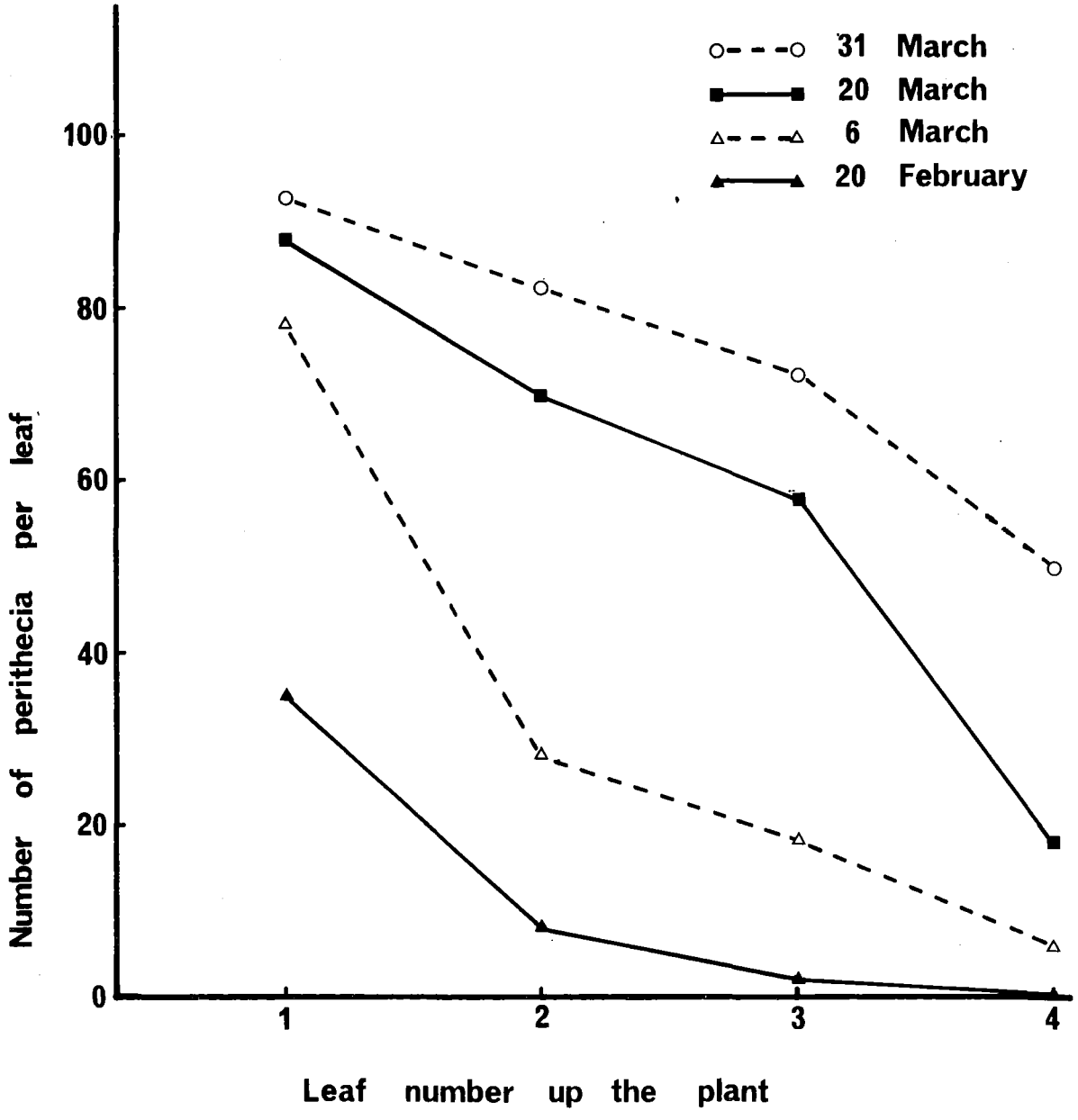
Month	% Germination	
	Incubation Temperature	
	24°C	10°C
January	96	-*
February	94	-
March	90	-
April	92	54
May	74	40
June	42	21
July	6	3

* not tested

TABLE 5: Germination, after 2 days on 2% water agar, of ascospores of *L. nodorum* and *Mycosphaerella* sp. obtained from perithecia collected from wheat stubble during the months April to July.

Month	% Germination	
	Incubation 24°C	Temperature 10°C
April	95	45
May	90	41
June	60	35
July	34	15

FIG. 2 Effect of age of wheat leaf tissue on appearance of perithecia of *L. nodorum* and *Mycosphaerella* sp. in wheat stubble during February/March of 1974.



5.3 ASCOSPORE DISPERSAL

5.31 Introduction

Septoria infection of wheat crops some distance from any known inoculum source of either *S. tritici* or *S. nodorum* has been attributed to wind dispersal of debris containing pycnidia (Weber, 1922), but the distance over which such inoculum is carried is unknown (Shipton et al., 1971). Wind dispersal of pycnidiospores over any distance has never been demonstrated, and Bockmann (1932) found little evidence for wind dispersal of *S. nodorum* in the field. Scharen (1966), working with *S. nodorum* under simulated wind and rain conditions, found the spread of spores was mainly by splash dispersal, and then only to nearby plants. Splash dispersal also occurs for *S. tritici*, but the distance over which this dispersal takes place is unknown (Shipton et al., 1971).

The sexual stages of *S. tritici* and *S. nodorum* have not received much attention, and so little is known about the liberation and dispersal of the ascospores of these two fungi. Sanderson (1974) trapped spores for a number of weeks during 1973 and found that spores of both *L. nodorum* and *Mycosphaerella* sp. were wind-borne, and that spore release appeared to be in response to rain, fog and dew.

After harvesting the field trial, (described in Section 6) a spore trap and rain gauge were established in wheat stubble to try and determine when ascospore liberation occurred, to confirm that the ascospores were windborne, and to try and correlate moisture and ascospore release.

5.32 Procedure

Most methods for trapping airborne spores are based on the impaction of spores on suitable adhesives (such as petroleum jelly or silicone grease) coated on microscope slides, plastic films, or metal rods. (Pady and Kelly, 1949; Hirst, 1952). However, there are drawbacks to the use of adhesives as it is difficult to achieve even coatings of adhesive or grease to prevent distortions of transmitted light during microscopic examination. McCoy and Dimock (1971) drew attention to the fact that greased slides are also difficult to stain. To overcome this difficulty, these authors used Scotch Brand Magic Transparent Tape No. 810, as the adhesive trapping substrate, and found that the tape did not lose its adhesive quality or ability to trap and retain spores in a number of environments. Spores on the tape were able to be stained without dislodging them.

For this study, a Burkard Recording Volumetric Spore Trap (Burkard Scientific (Sales) Ltd, England) was used. This spore trap is a modification of the Hirst Trap (Hirst, 1952), and consists of a drum with a circumference of 345 mm placed behind a critical orifice 2 mm wide x 14 mm long. Air was drawn through the orifice by a vacuum pump, (powered by a 6 volt battery), which had a maximum flow rate of 10 litres/minute (0.6 m^3/hr.). Scotch Brand Magic Transparent Tape No. 810 (Minnesota Mining and Manufacturing (Aust.) Pty.), 19 mm wide, was wrapped, sticky side out around the drum, and attached to it by a strip of double-sided sticky tape.

The trap was placed in the centre of the area of stubble, so that spores from prevailing wind directions could be carried through the slit and impacted onto the tape, approximately 2 mm of which moved past the orifice each hour. The drum was set for one complete rotation per week. At the end of a run, the tape was removed from the drum and transported back to the laboratory in a dust-free container. The tape was then removed from the drum and fixed onto a perspex block by means of a small piece of double sided sticky tape at each end. The block was marked at 48 mm intervals, representing lengths of 24 hours. Care was taken during this procedure to see that the tape was correctly orientated, and that the starting point was on the left side of the perspex block. Once in position, the tape could be cut into daily sections.

Sanderson (1974) advised that staining of the tape to reveal spores was not necessary, so the tapes were dipped into 75% ethanol and placed sticky side down on a glass coverslip, 24 x 60 mm. As the ethanol evaporated, the adhesive of the tape bonded to the coverslip. Bubbles and excess ethanol were squeezed out with a tissue before the tape dried. When dry, the coverslip was permanently mounted on a slide with 'Glyceel' mounting medium (Searle Scientific Services, England).

The number of spores was counted using a microscope with a mechanical stage and vernier scales calibrated in millimetres. A magnification of x500 was used, with a blue filter to increase the contrast of the hyaline ascospores. A traverse, 500 microns wide was made parallel to the tape movement, with the number of spores counted over 2 mm being the number per hour. In actual fact, this represented only $^{1}/_{28th}$ of the total catch per hour. Efficiency of the trap is given by the manufacturers as 70 $\pm$ 20%. The numbers thus counted are below the true value, but the technique provides a relative index of spores liberated.

Hirst (1953) has shown that the presence of ascospores in the air spora is influenced by moisture. A Cassella rain gauge (Cassella, London) was placed adjacent to the spore trap. This had a recording chart on a drum which completed one revolution per week. Rainfall data could then be compared with the spore catch to see if there was any correlation.

Results.

Spore trapping data showed that the ascospores of *L. nodorum* and *Mycosphaerella* sp. were part of what Hirst (1953) called the 'damp air' spora. Within a few hours of rain, spores of both types were trapped in relatively large numbers (Fig. 3), and this pattern occurred throughout the length of time the spore trap was in operation. In the absence of rain, spore release occurred only at night (Fig. 4), most spores being trapped between midnight and dawn. Hirst (1953) believed that this release was in response to dew formation at night.

FIG. 3 The effect of rainfall on the release of ascospores of *L. nodorum* and *Mycosphaerella* sp. from perithecia in wheat stubble during mid-March, 1974.

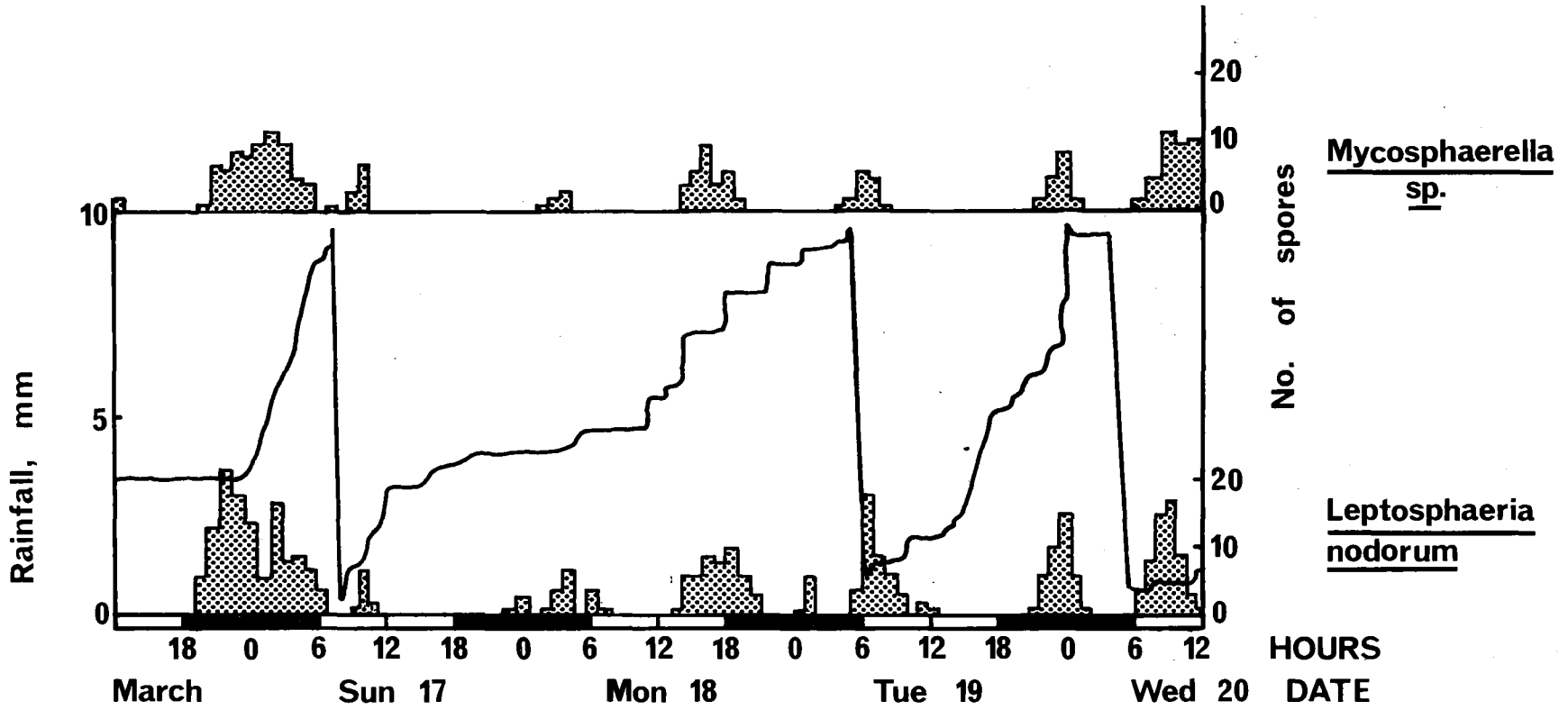
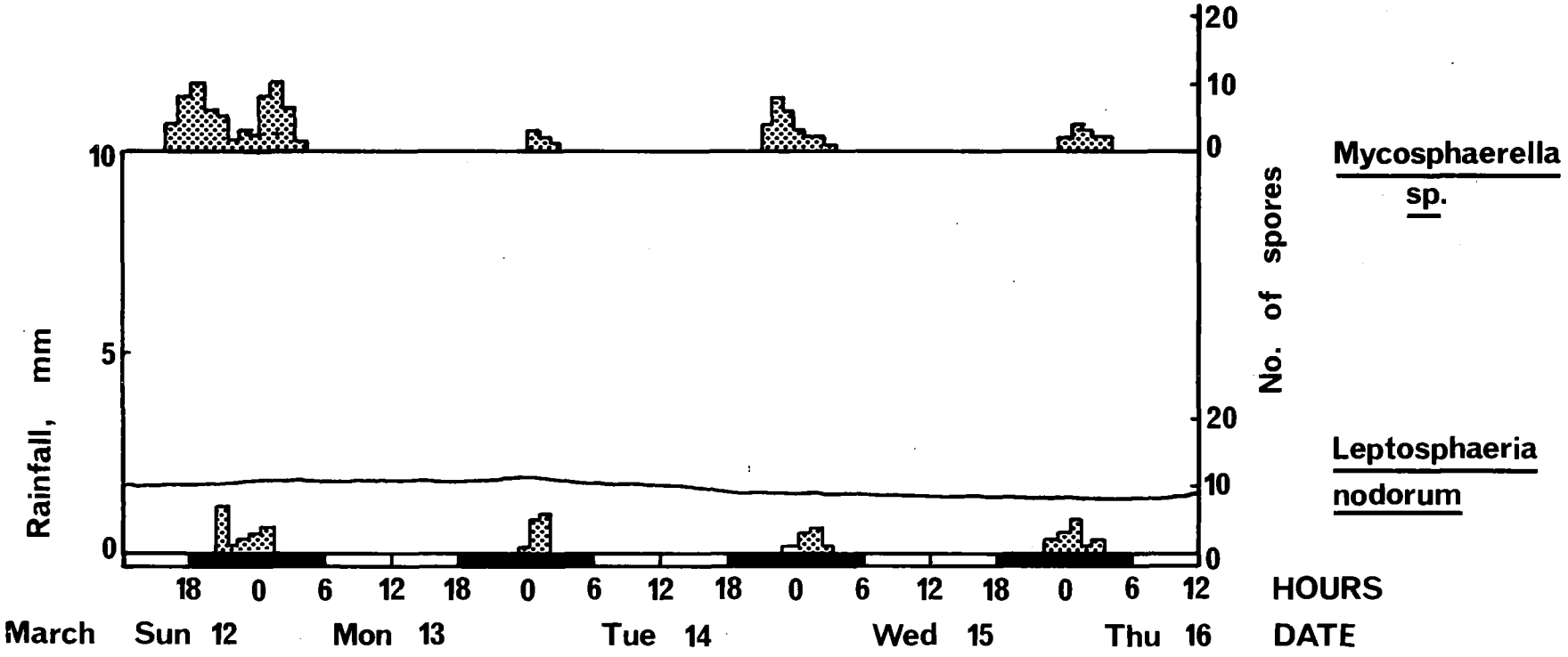


FIG. 4 The release of ascospores of *L. nodorum* and *Mycosphaerella* sp. from perithecia in wheat stubble in the absence of rain, March 1974.



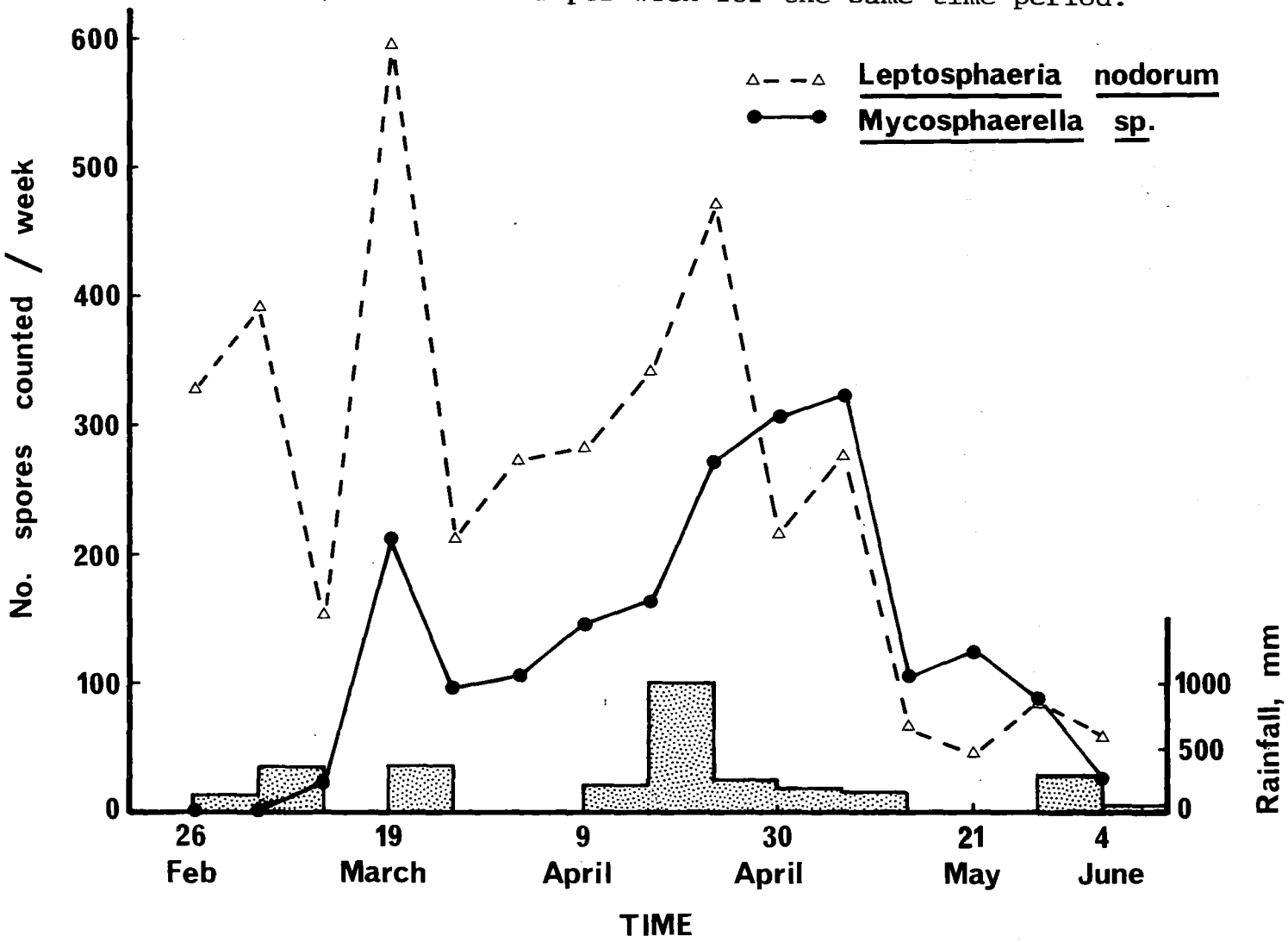
In order to obtain a complete picture of spore dispersal, traps would have to be established at sites some distance from wheat stubble in order to determine how far the ascospores were carried in the wind. This was not possible to do, and in most years would be difficult because of the many areas of wheat stubble in Canterbury. However, Sanderson (1972b) stated that ascospores of *L. nodorum* and *Mycosphaerella* sp. can be transported many miles by the wind.

5.33 Ascospore release - February to June, 1974

Although the counting of spores was not strictly quantitative it does provide an index of spore release as a function of time (Fig. 5). This data is a summation of the numbers of spores of each type trapped per week, and the total rainfall per week, which influences the number of spores released. Ascospore release occurred mainly through March-May, and by June had declined. Ascospores of *L. nodorum* were being released 2 weeks before those of the *Mycosphaerella* sp., and this is because the perithecia of *L. nodorum* were mature earlier than those of the *Mycosphaerella* sp. (see Section 5.21).

Fig. 5 shows that ascospores were being released at a time (May), when many autumn sown wheat crops were starting to grow, and thus initial infection in these crops could be from airborne ascospores, especially in those crops not in contact with other sources of primary inoculum. In this way, the sexual stages of the *Septoria* fungi could play an important part in the epidemiology of the *Septoria* diseases.

FIG. 5 Numbers of ascospores of *L. nodorum* and *Mycosphaerella* sp. released from perithecia in stubble and trapped per week from February to June 1974; and rainfall per week for the same time period.



5.4 SEED BORNE INOCULUM

5.41 Introduction

Seed-borne infection of *S. tritici* has not been demonstrated (Shipton et al., 1971), but seed-borne infection of *S. nodorum* is well documented. It has been found in Canadian wheat samples, (Machacek, 1945) and in Australian samples (Shipton and Chambers, 1966), while Hewett (1965) believed that seed must be an important source of the disease in Great Britain. These and other reports suggest that seed-borne *S. nodorum* can be an important inoculum source. As a consequence, samples of New Zealand wheat seed were tested for *S. nodorum*.

5.42 Procedure

Two methods were used to detect the presence of *S. nodorum* in seed.

a) Kietreiber Test (Kietreiber, 1961).

Wheat seeds were placed between damp blotters, left for 14 days at 10°C in the dark, and then inspected for signs of infection, such as discrete brown spots on the coleoptile, twisting of the seedling, a shortened coleoptile, and small brown protuberances on the shoot.

b) Agar plate (Hewett, 1965).

Wheat seeds were surface sterilized in a solution containing 0.5% available chlorine for 10 minutes, and then placed on the surface of PDA, 10 seeds per 9.5 cm petri dish. The dishes were then incubated at 24°C in the dark and examined on the fifth day.

Results.

Five lines from Canterbury of each of the wheat varieties Aotea, Arawa and Hilgendorf were tested by both methods: 100 seeds per test for the Kietreiber method, and 200 seeds per test for the agar plate method.

In the Kietreiber test, the seedlings did not develop any of the symptoms typical of *S. nodorum* infection. Coleoptiles grew straight and were unmarked. Some showed signs of saprophytic fungal growth, but *S. nodorum* was not detected.

In the agar plate test, fungal colonies grew from most seeds, and initially a number of these were thought to be *S. nodorum*. However, sub-cultures from these colonies showed that colonies, which in a young stage looked similar to *S. nodorum*, were in fact colonies of either a *Fusarium* sp. or an *Alternaria* sp.

S. nodorum was not detected in these tests on seed. This is perhaps to be expected, for as yet in New Zealand, *S. nodorum* has been recorded only on the leaves and not the heads of wheat plants. The evidence suggests that *S. nodorum* is not seed-borne in New Zealand, but more extensive tests of all varieties grown in New Zealand would have to be undertaken in order to determine this conclusively.

5.5 DISCUSSION

The importance of both *Leptosphaeria nodorum* and *Mycosphaerella* sp. in providing primary inoculum (ascospores), for the *Septoria* diseases of wheat has become evident. The fact that they are wind-borne means that infection can be initiated in autumn-sown crops some distance from other kinds of inoculum.

Viable pycnidiospores of *Septoria tritici* were present on stubble and on volunteer plants throughout the autumn and early winter. These spores can provide primary inoculum only over a very limited area, for they are not wind-borne. However, plant debris containing pycnidia could be blown into new crops, and thus also provide an inoculum source. *S. nodorum* pycnidia were not found at this time, and the reason for this is still not clear.

In New Zealand, *S. nodorum* does not appear to be seed-borne, and it is unlikely that infection would be initiated in this way.

C H A P T E R VI

CONTROL

6.1 INTRODUCTION

Methods advocated for the control of *Septoria* diseases of cereals are: cultural; the use of resistant varieties; foliar spraying; seed treatments; and quarantine. (Shipton et al., 1971). Of these, cultural practices such as removal or destruction of stubble and volunteer plants which could provide sources of primary inoculum, are essential in the overall control programme. In the long term, control through the use of resistant or tolerant varieties would be the best approach. Seed treatment has given variable results for control of *Septoria nodorum*, and as yet, *S. nodorum* has not been found to be seed-borne in New Zealand.

The value of foliar spraying has been debated, for many consider this an uneconomic approach. (Shipton et al., 1971). However, there has been little work on this aspect of control, although Shipton (1968), Jenkins and Morgan (1969), and Melville and Jemmett (1971), did achieve reasonable control by spraying with dithiocarbamates, but with uneconomical and impractical spraying schedules. In New Zealand, Sanderson (1964b) obtained a ten per cent yield increase after spraying with captan and zineb throughout the growing season to control *Septoria tritici*.

In this study, two aspects of control, foliar spraying and host resistance were investigated.

6.2 FOLIAR SPRAYING

6.21 Introduction

As soon as *Septoria* fungi have become established in a crop, there is a potential for rapid spread of the disease through the crop. In an effort to prevent this, spray or dust treatments can be applied to eradicate the pathogens, or to protect the healthy plants. When economics allow, a series of treatments can be planned and applied to protect plants during all potentially infective periods. The efficiency of any treatment will depend on the persistance of the fungicide between one treatment and the next, and the distribution of the fungicide during the infective periods (Evans, 1972).

Under New Zealand conditions, potentially infective periods can exist right throughout the growing season, from the seedling stage to flowering, so that it is difficult to decide when to apply any form of treatment. The results of Jenkins and Morgan (1969) and Shipton et al. (1971) suggest that attacks by *Septoria* fungi at tillering and ear emergence appear to cause the greatest reduction in yields. Therefore, the application of sprays to these growth stages was investigated.

6.22 Fungicides Used

a) Benomyl: A protective and eradicant fungicide with systemic activity, introduced by E.I. du Pont de Nemours and Co. in 1967. (Delp and Klöpping, 1968). The chemical name is methyl N-(1-(butylcarbamoyl)-2-benzimidazole) carbamate, and its structure is shown in Fig. 6. Benomyl is formulated as a 50% wettable powder, and marketed as 'Benlate'.

b) Thiophanate-methyl: A systemic fungicide, introduced in 1969 by the Nippon Soda Co. Ltd (Aelbers, 1970). This compound is 1,2-di-(3-methoxycarbonyl-2-thioureido) benzene (Fig. 6), and is formulated as an 80% wettable powder (Topsin M), for spray applications.

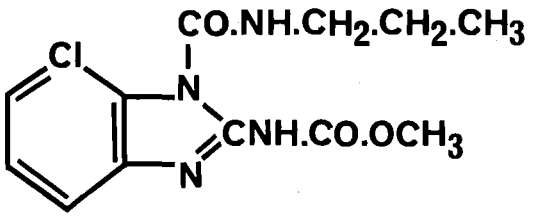
c) Mancozeb: A protective fungicide, introduced in 1961 by the Rohm and Haas Co. Ltd. It is a complex of zinc and maneb (manganese salt of ethylene bis (dithiocarbomic) acid), containing 20% manganese and 2.5% zinc. The product is formulated as a wettable powder, often containing at least 80% w/w mancozeb, and marketed by Rohm and Haas under the trade name 'Dithane M45'.

6.23 Procedure

In 1973, a trial on the Lincoln College Research Farm evaluated the effectiveness of chemical sprays against the *Septoria* diseases of wheat, and tried to determine at which growth stage of the plant the maximum benefit from spraying could be obtained. Wheat, (cv. Aotea), was sown at 100 kg/ha

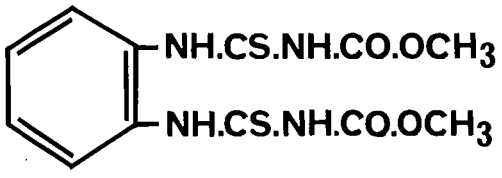
FIG. 6 STRUCTURAL FORMULAE OF SPRAY CHEMICALS

a)



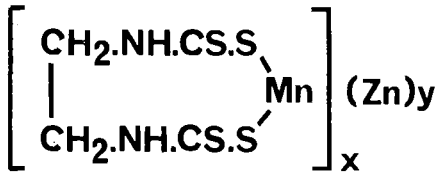
methyl N-[1-(butylcarbamoyl)-2-benzimidazole] carbamate
Benomyl

b)



1,2-di-(3-methoxycarbonyl-2-thioureido) benzene
Thiophanate

c)



complex of zinc, manganese and the ethylene bis(dithiocarbamate) ion
Mancozeb

on the 5th of July 1973, and 6 x 4 m plots were established within the crop. Each plot was separated by a 2 metre border of Aotea wheat.

The materials used: benomyl, mancozeb and thiophanate-methyl, were all applied at standard rates as follows:

TABLE 6: Sprays applied to Aotea wheat to control *Septoria tritici* diseases.

Treatment	No. of Sprays	Rate	Date of Spraying	Growth Stage of Plant at Spraying*
benomyl	1	0.27 kg/ha	18/9/73	G.S.3
benomyl	3	0.27 kg/ha	18/9/73 11/10/73 23/11/73	G.S.3 G.S.7 G.S.10.1
mancozeb	2	2.17 kg/ha	20/9/73 23/11/73	G.S.3 G.S.10.1
thiophanate-methyl	1	0.54 kg/ha	20/9/73	G.S.3

* For explanation of growth stage of the wheat plant, see Fig. 7.

These treatments, plus a non-treated plot were replicated five times and randomized within the trial. Sprays were applied with a knapsack-type power sprayer (Maruyama Mfg. Co. Ltd, Japan), using hollow cone nozzles with a volume rate of 222 litres water/ha.

FIG. 7. Growth stages in cereals, (Feekes - Large scale).

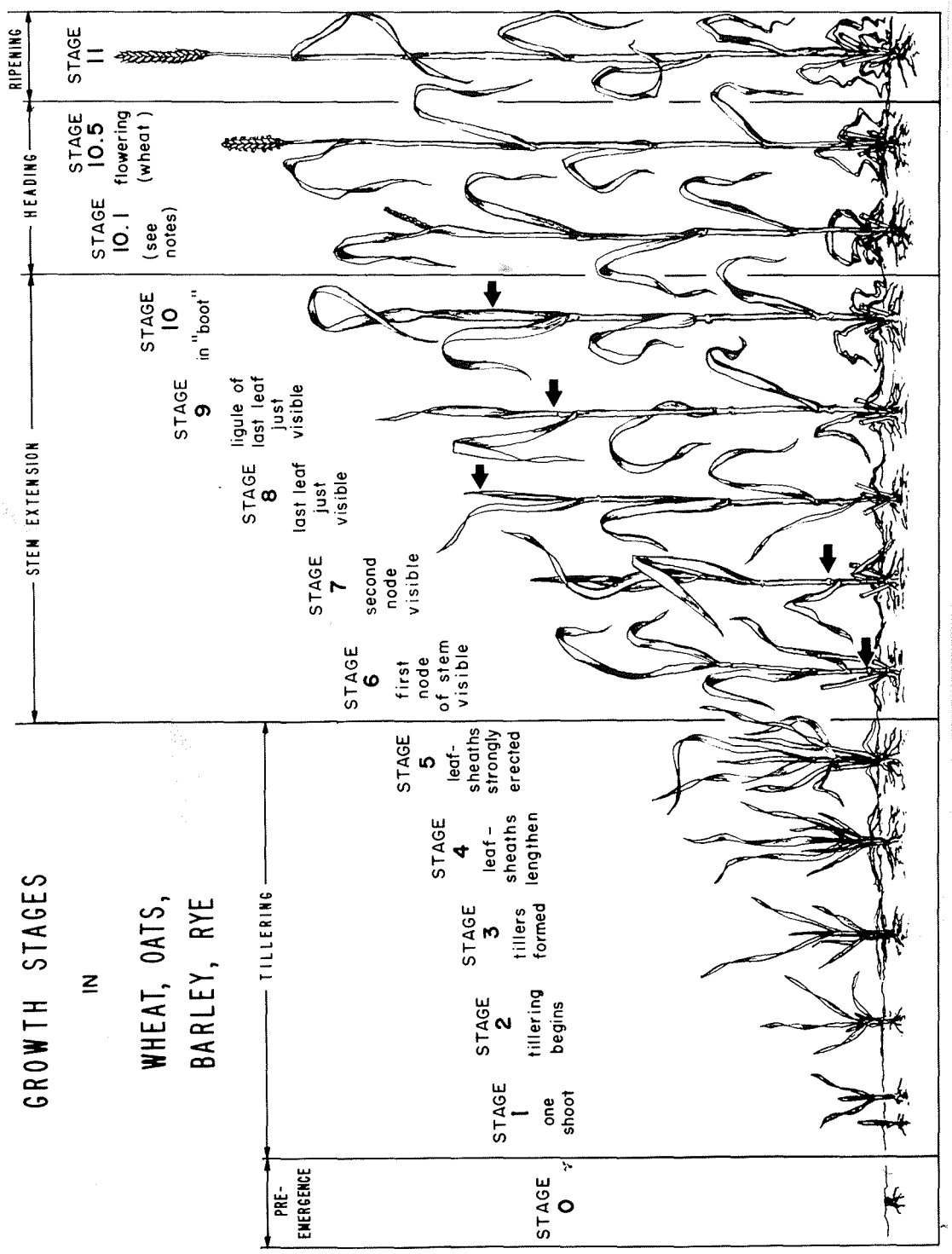
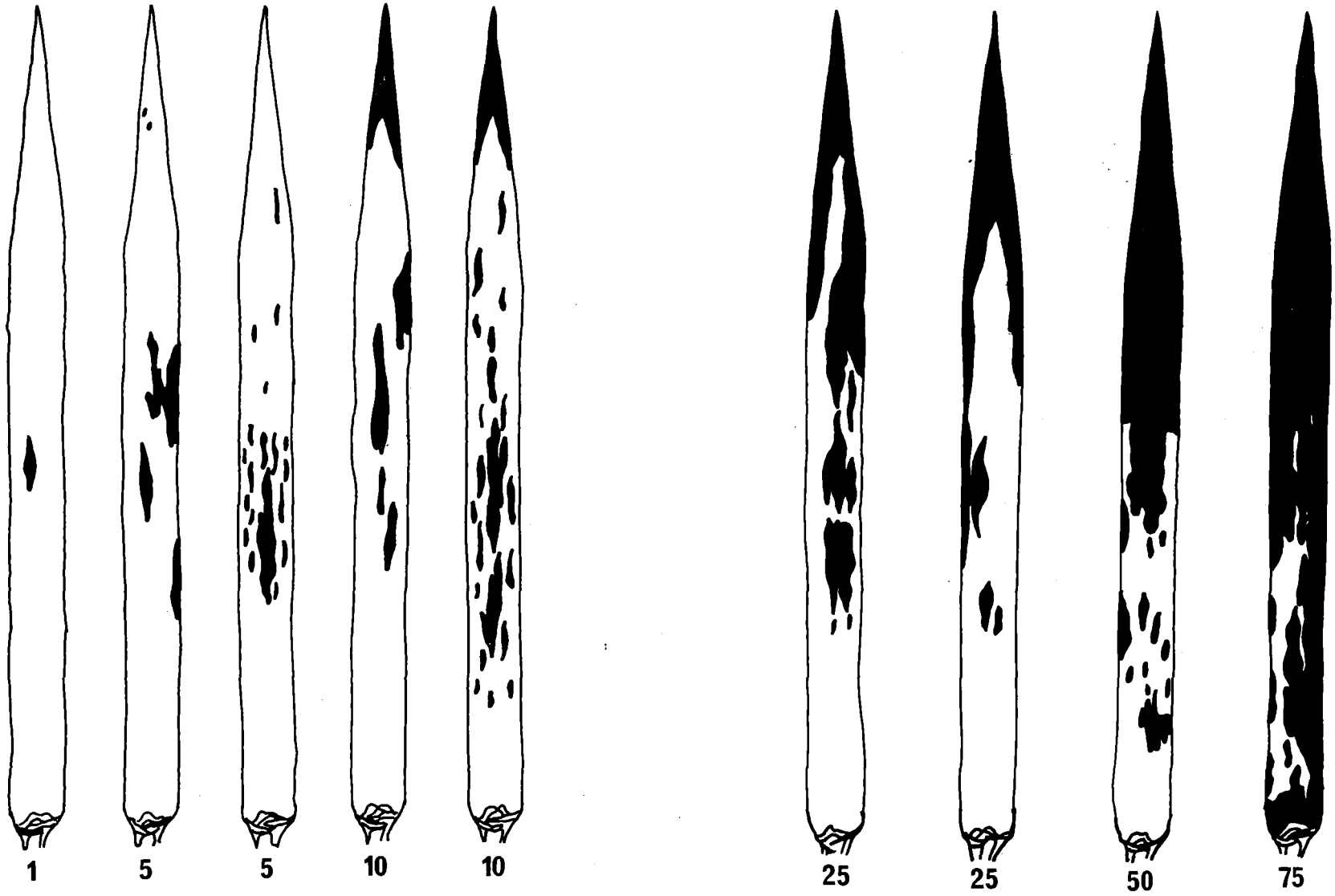


FIG. 8 Key for assessment of *Septoria* infection as a percentage of leaf area (Anon, 1968)



Throughout the growing season, plots were assessed for the severity of *S. tritici* and *S. nodorum* on the leaves, and at maturity, on the heads, using provisional standard keys (Fig. 8) devised by the Plant Pathology Laboratory, Harpenden (Anon, 1968). Disease was assessed on those leaves recommended (Anon, 1968) and these were as follows:

For recording:

a) disease incidence up to G.S. 6; infection was assessed on the lowest green leaf.

b) disease development and early differences in susceptibility from G.S. 7 to G.S. 10.5; infection was assessed on leaf 3 - from G.S. 7 to G.S. 8, leaf 3 arises from the second lowest node; from G.S. 9, it is the third from the top of the plant.

c) infection likely to be related to loss in yield; infection was assessed at G.S. 11.1 on leaf 2 - leaf immediately below the flag leaf.

From each plot, ten fertile tillers were selected at random and *Septoria* on certain leaves assessed. Any other leaf diseases present were also recorded, as was the percentage of the leaf lamina remaining green.

At harvest, (12th February, 1974), plots 1 m^2 were cut and bundled into sheaves. From a sample, the number of grains per ear and stem length were recorded, and then each sheaf was individually thrashed, yield/m^2 obtained, and 1000 grain weights determined. The remainder of each plot was harvested by a header with a one metre cut, and bulk plot yields obtained.

6.24 Results

Septoria tritici was present on the lower leaves when spraying began in September, its severity increased and eventually 35% of the area of the flag leaf in the unsprayed treatment was affected. *S. nodorum* was not found on the leaves throughout the growing season, and neither *S. tritici* or *S. nodorum* was found on the heads.

Two other leaf diseases, powdery mildew (*Erysiphe graminis* D.C.) and leaf rust (*Puccninia recondita* Rob. ex Desm.) occurred in the trial. On the flag leaf in all plots, only traces of mildew were recorded, but 5% of the leaf areas was affected by leaf rust.

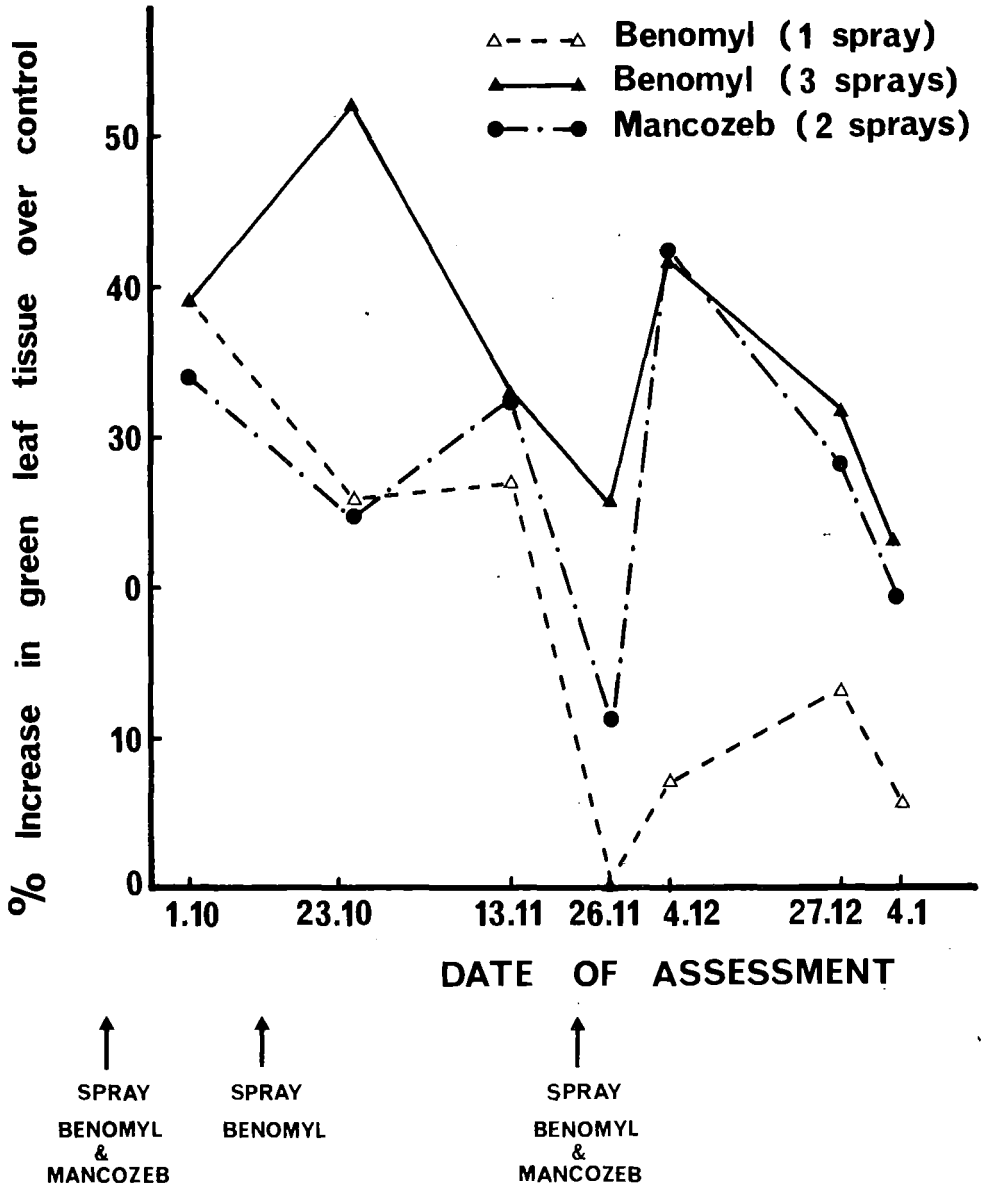
a) Disease Assessment

When applied at tillering (G.S. 3), all three fungicides were partially effective (Table 7), but this effect lasted only for six weeks, by which stage there was little difference between untreated and treated plots (Fig. 9).

TABLE 7: Effect of Fungicide Sprays at Tillering (G.S. 3) on Severity of *S. tritici* infection.

	% Infection Leaf 3*		
No. days after spraying	12	56	65
Growth stage of crop	G.S. 5	G.S. 10.1	G.S. 10.5.1
untreated	26.8	44.5	56.1
benomyl	10.5	33.4	48.2
mancozeb	12.1	34.3	47.9
thiophanate-methyl	9.9	35.0	49.2

FIG. 9. Effect of sprays of benomyl (once and three times) and mancozeb (twice) during the growing season, on control of *S. tritici* on leaf 2 - expressed as % increase in green leaf tissue over that of unsprayed plants.



* at G.S. 5, leaf 3 is the lowest green leaf; at G.S. 10.1 and G.S. 10.5.1, leaf 3 is the third from the top of the plant.

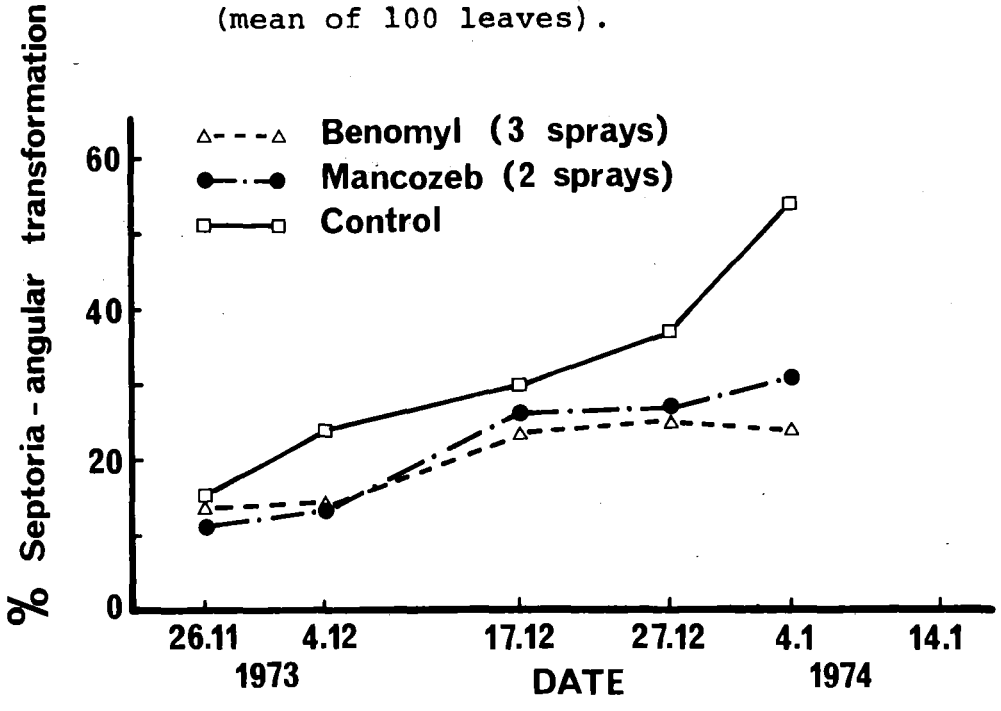
Benomyl and mancozeb, when applied at ear emergence (G.S. 10.1), reduced the severity of *S. tritici* on both the flag leaf and the one immediately below (leaf 2) (Table 8).

The main effect of spraying at ear emergence was to increase the persistance of green tissue in both the flag and second leaves (Figs. 10 and 11), and this largely reflects disease control.

A summary of the effect of fungicide spraying on the amount of green leaf tissue of the wheat plant during the growing season is seen in Fig. 9. A single spray of either benomyl or mancozeb effectively controlled the disease, as indicated by the greater amount of green tissue in treated as compared with untreated plots. This control lasted for a period of between four and five weeks, after which the disease began to increase in incidence. If the aim of spraying is to control *S. tritici* throughout the entire growing season, then the results suggest that, in a season favourable to *S. tritici*, more than one spray may have to be applied to successfully achieve control.

FIG. 10.

a) Effect of fungicide spray at G.S. 10.1 on the amount of *S. tritici* infection on leaf 2 of the wheat plant (mean of 100 leaves).



b) Effect of fungicide sprays at G.S. 10.1 on the percentage of green tissue on leaf 2 of the wheat plant (mean of 100 leaves).

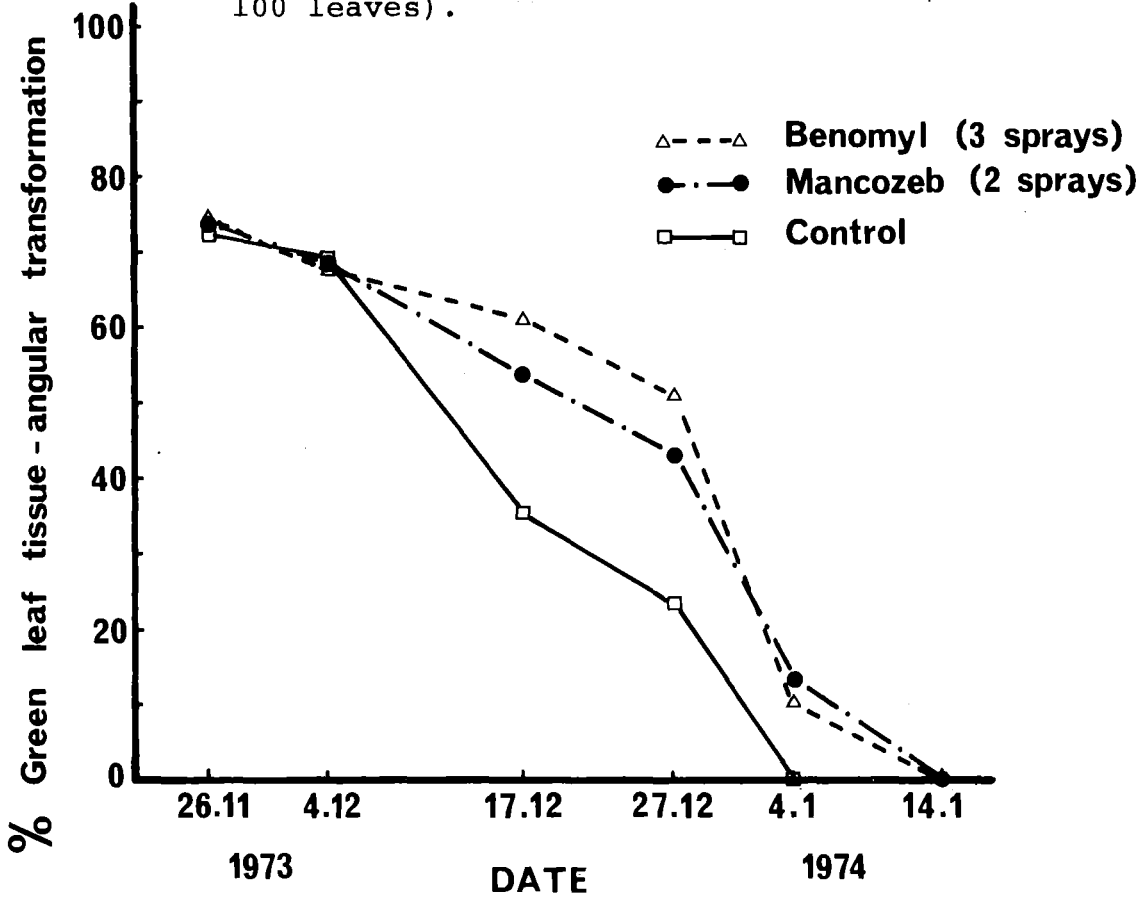
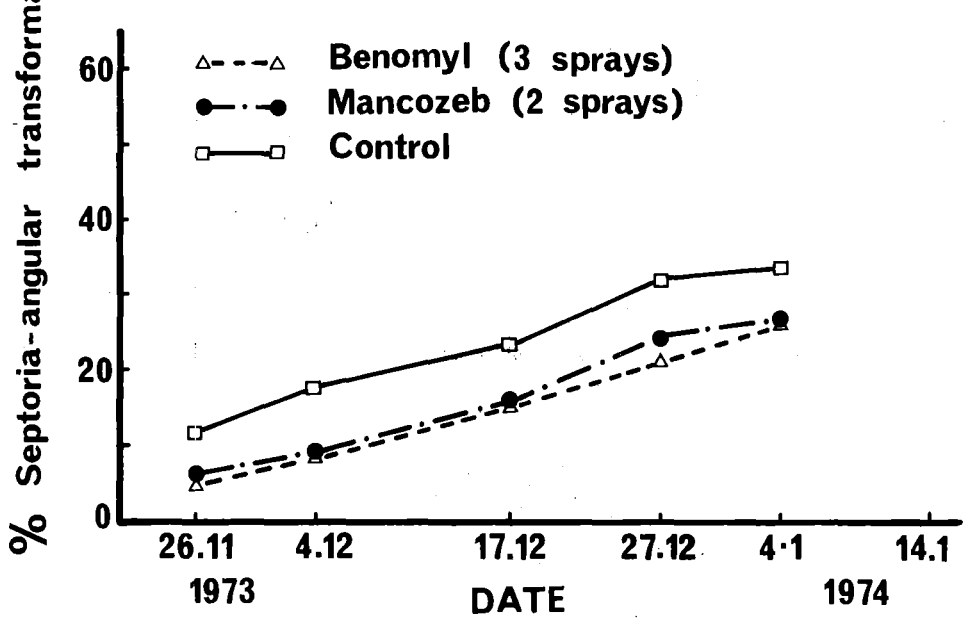


FIG. 11.

a) Effect of fungicide sprays at G.S. 10.1 on the amount of *S. tritici* infection on the flag leaf of the wheat plant (mean of 100 leaves).



b) Effect of fungicide sprays at G.S. 10.1 on the percentage of green tissue on the flag leaf of the wheat plant (mean of 100 leaves).

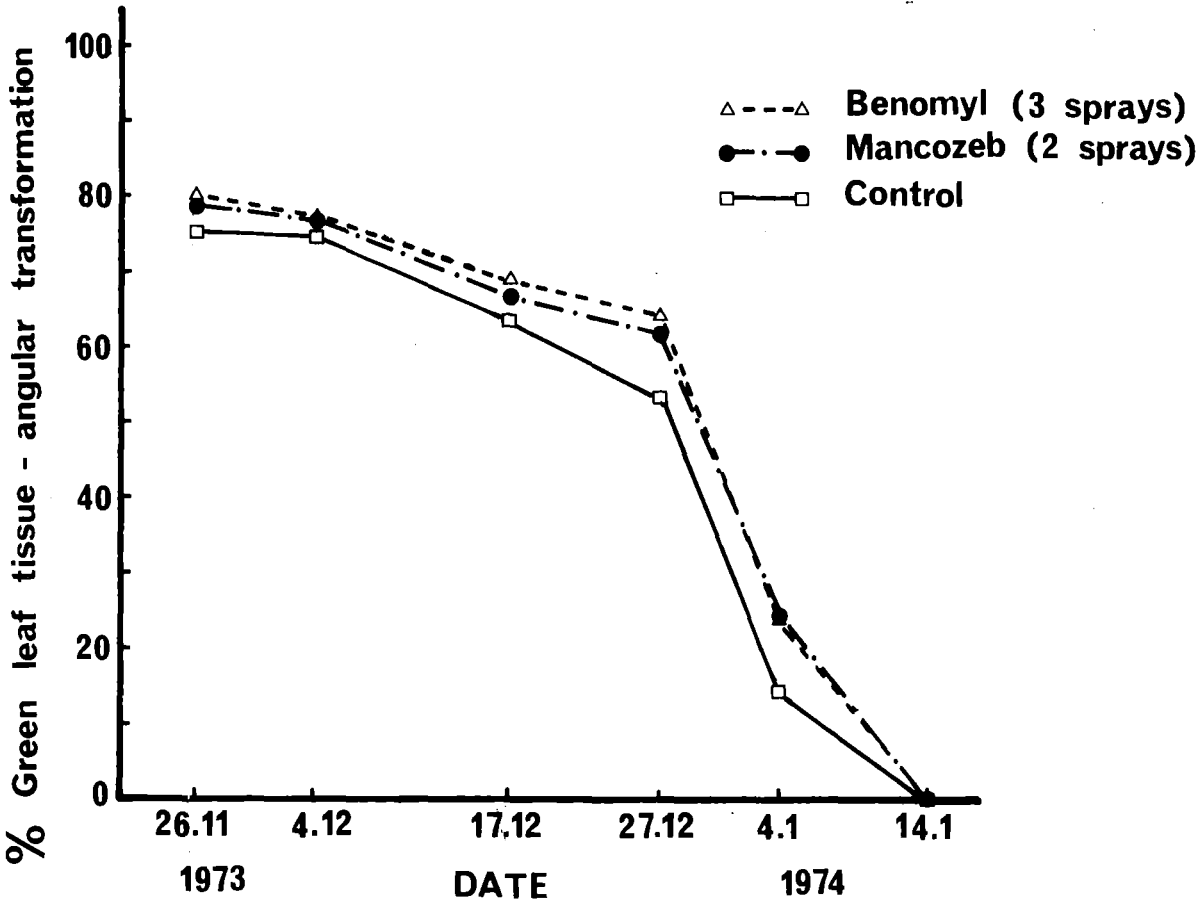


TABLE 8: Effect of Fungicide Sprays at Ear Emergence (G.S. 10.1) on Severity of *S. tritici* infection.

% Infection on Flag Leaf and Leaf 2*							
Days after Spraying	Growth stage of crop	Untreated		Benomyl		Mancozeb	
		Flag	Leaf 2	Flag	Leaf 2	Flag	Leaf 2
11	10.5.3	9.1	16.5	2.2	5.7	2.6	5.6
24	11.1	16.0	24.5	6.9	15.8	7.1	19.0
34	11.1	28.0	36.7	13.1	16.8	17.2	20.1
42	11.2	31.2	66.9	19.5	18.4	20.3	26.5

* Leaf 2 is the leaf immediately below the flag leaf.

b) Yield Data

Table 9 shows that a single spray of benomyl or thiophanate-methyl had no effect on any of the yield parameters measured, but a spraying regime which included three sprays of benomyl or two of mancozeb gave significant increases (at 5% level) in 1000 grain weight, and for the benomyl treatments, in number of grains per ear (5% level) (Appendix 1). There was an increase in yield/m^2 but this was not significant. Bulk yields per plot were not significantly increased. There was a significant increase (1% level) in ear length with benomyl applied at G.S. 10.1, but the reason for this increase is not clear. Plant height was not affected by any treatment.

6.25 Discussion

Spraying mancozeb or benomyl at tillering (G.S. 3) as well as at ear emergence (G.S. 10.1) increased grain yield

TABLE 9. Effect of Fungicide Sprays on Yield Parameters

Treatment	Total Yield (g/m^2)	% Increase over untreated plots	1000 grain weight (g)	Grains per Ear	Ear length (cm)	Plant Height (cm)
untreated	261.4	-	39.1	23.6	6.5	55.5
thiophanate-methyl (one spray)	278.6	6.6	39.5	23.7	6.6	55.4
benomyl (one spray)	281.4	7.6	41.2	24.9	6.7	56.2
mancozeb (two sprays)	298.0	13.9	41.4*	25.8	6.8	56.6
benomyl (three sprays)	305.8	16.9	42.2**	29.3*	7.2**	58.4
S.E. (treatment mean)	8.59		0.02	1.12	0.87	0.83

* significant difference from unsprayed plots at P = 0.05

** " " " " " " P = 0.01

by 14 and 17% respectively. If it is assumed that all the increase in yield was due to disease control, then *S. tritici*, in this experiment, caused a loss in yield of about 11%. However, control was not complete, and so the loss in yield, due to this disease is likely to be greater.

It is possible that the fungicides influenced yields in ways other than by disease control, such as by affecting the physiology of the host; modifying the leaf microflora; or through nutritional effects. It seems likely that the increases in 1000 grain weight were mainly due to reduction of *S. tritici* attack on the upper part of the plant. Although leaf rust did occur in this trial, there was no difference in severity of attack between treated and untreated plots, and so it can be assumed that if yield was affected by leaf rust, it had the same effect on all treatments.

The effect of spraying was to increase the persistance of green leaf tissue. Grain size is primarily a function of that portion of the plant above the flag leaf node, and the duration over which it remains photosynthetic (Thorne, 1965). In this trial, both benomyl and mancozeb delayed senescence of both leaf 2 and the flag leaf, and this is reflected by the increase in 1000 grain weight by these two treatments. Similar results were obtained by Shipton (1968) and Jenkins and Morgan (1969) for an epidemic in which both *Septoria* species were present, and by Melville and Jemmett (1971) who found *S. nodorum* infection in the head as well as in the leaves of the plant.

Results from the spray trial suggest that *S. tritici* infection of wheat crops can cause significant yield reductions, mainly through increasing the rate of senescence of leaf 2 and the flag leaf. Control of the disease was best achieved by the use of a fungicide spray at ear emergence (G.S. 10.1), rather than at tillering (G.S. 3). However the best time for application of the spray was not conclusively determined, and further trial work would be needed to investigate this aspect.

6.3 HOST RESISTANCE

6.31 Introduction

Heritable resistance to *S. tritici* has been clearly demonstrated (Mackie, 1929; Morales, 1957) and is governed by either a single dominant or recessive gene.

Although there are a number of other references on varietal resistance to *S. tritici*, Shipton et al. (1971) consider that much may be of questionable value, as the conditions governing infection and symptom expression may not have been fully appreciated. However, resistant varieties would be of value, and an effective method of controlling the disease caused by *S. tritici*. A breeding programme would be needed to select for resistance factors, and to incorporate them in with other resistance and agronomic factors.

With *S. nodorum*, true resistance has not been demonstrated. Some varieties do possess a degree of tolerance, but there have been no convincing demonstrations that the tolerance observed was heritable (Shipton et al., 1971).

Host resistance to *Septoria* diseases of wheat has not received much attention, and little is known of the reactions of New Zealand wheat varieties to these diseases.

During the 1973/74 season, observations and disease assessments were made in the field on New Zealand wheat varieties. In the laboratory, the leaves of a number of wheat varieties were inoculated under a controlled set of conditions, to see if varieties showing resistance in the field also did so under conditions more favourable to the pathogens.

6.32 Field Observations

During the 1973 season, wheat variety trials both at Lincoln College and D.S.I.R., Lincoln were assessed for *Septoria* infection, by the method described in Section 6.23. All infection was natural, and was found to be caused by *S. tritici*. *S. nodorum* was not detected.

The trial at D.S.I.R., Lincoln, involved six varieties; Aotea, Arawa, Hilgendorf, Cross 7, Kopara and Gamenya. Plots measured 2 x 1 m, and each variety was replicated twice within two blocks. Assessments were made on 25 leaves per plot at G.S. 7 (assessment of leaf 3) and G.S. 11.1 (assessment of leaf 2) (Anon, 1968).

The trial at Lincoln College was a variety x sowing date trial. There were four sowing dates (March, April, May and June), and five varieties (Aotea, Arawa, Hilgendorf, Karamu and Kopara). Plots measured 3 x 30 m. Each treatment was randomized in each of three blocks. Twenty-five leaves from each plot were assessed at different growth stages throughout the growing period.

6.33 Results

Results of assessment of the D.S.I.R. trial are shown in Fig. 12. At G.S. 7, varieties Aotea, Gamenya, and Cross 7 showed significantly more infection (1% level) by *S. tritici* than varieties Arawa and Hilgendorf, and this was also the case at G.S. 11.1 (Appendix 2). However it should be noted that although varieties Aotea and Hilgendorf showed more resistance than the other varieties, they still had 25% infection on leaf 2 at G.S. 11.1.

At Lincoln College, the variety Aotea showed significantly more infection (1% level) than any other variety (Fig. 13; Appendix 2). Time of sowing had no effect on the amount of *S. tritici* infection on any variety (Fig. 14).

After harvest, ten bottom leaves per plot in one block of the stubble of this trial were examined for perithecia of *Mycosphaerella* sp. and counts showed that the variety Aotea contained significantly more perithecia per leaf than the other varieties (Table 10). This result was to be expected, as at G.S. 11.1, variety Aotea showed more *Septoria* infection than any other variety.

FIG. 12. The amount of *S. tritici* infection on leaf 3 (G.S. 7) and leaf 2 (G.S. 11.1) on six wheat varieties from 1974 D.S.I.R. variety trials.

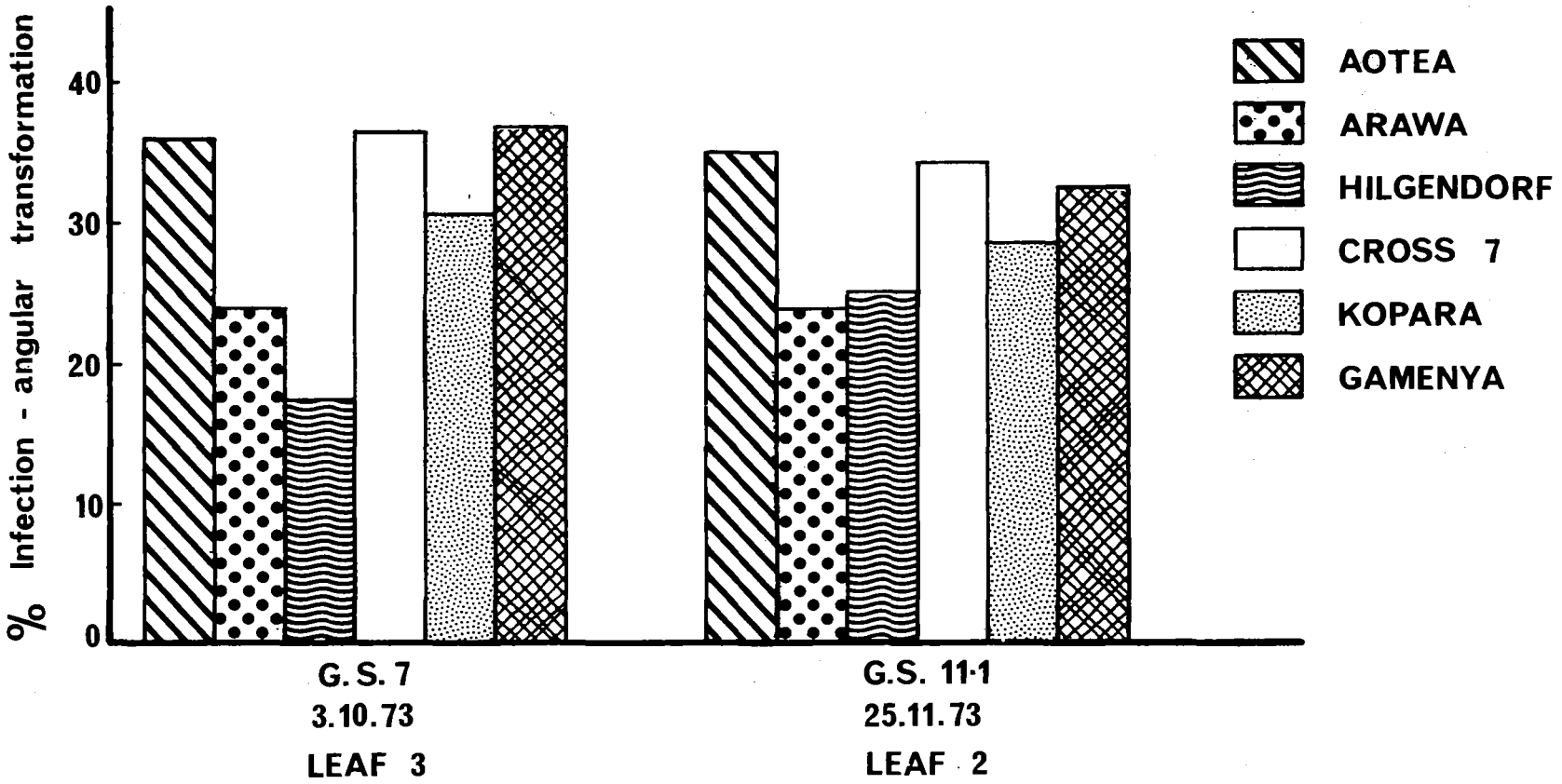


FIG. 13. The amount of *S. tritici* infection on leaf 2 (G.S. 11.1) on five wheat varieties, each with four sowing dates.

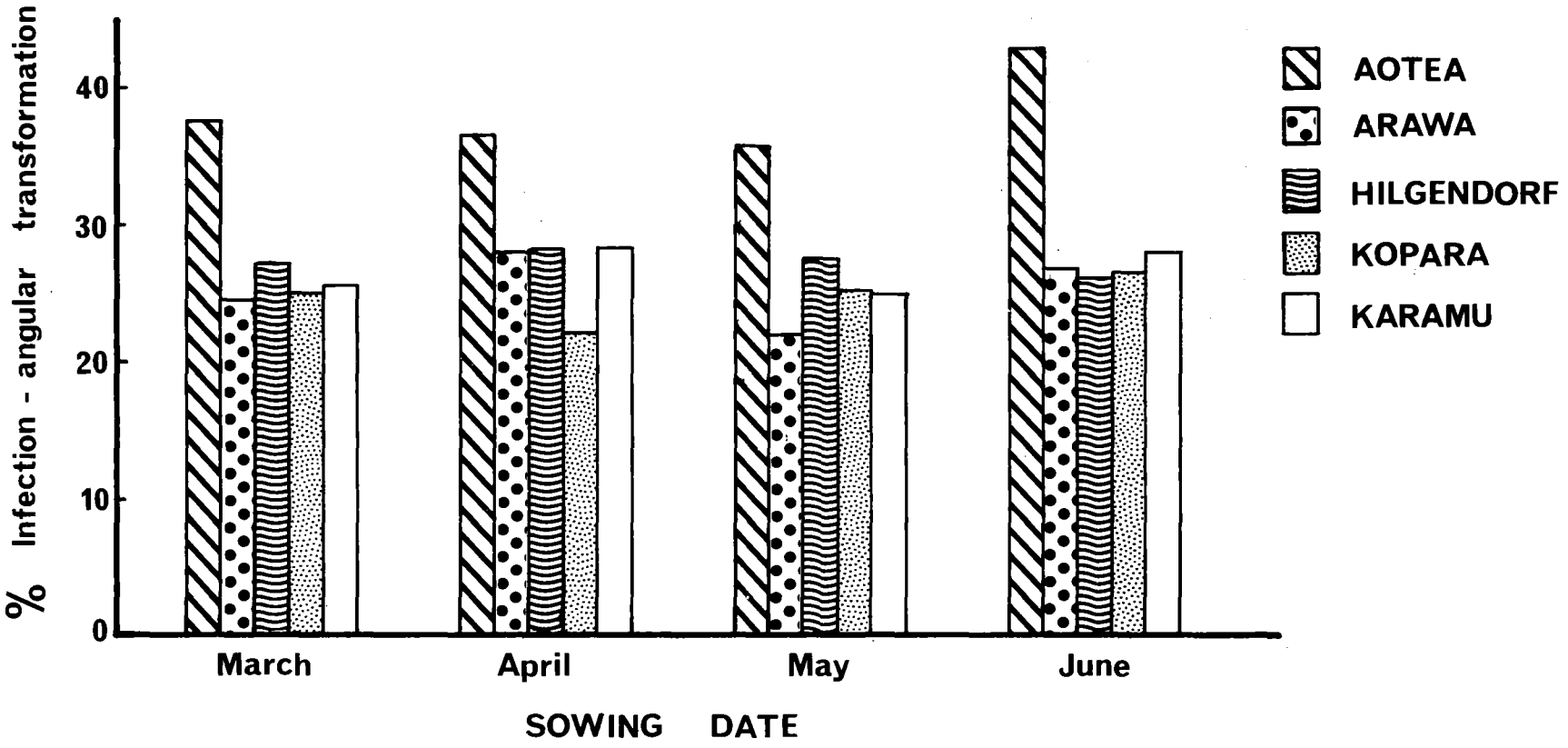


FIG. 14. The effect of date of sowing of five wheat varieties on amount of *S. tritici* infection on leaf 2.

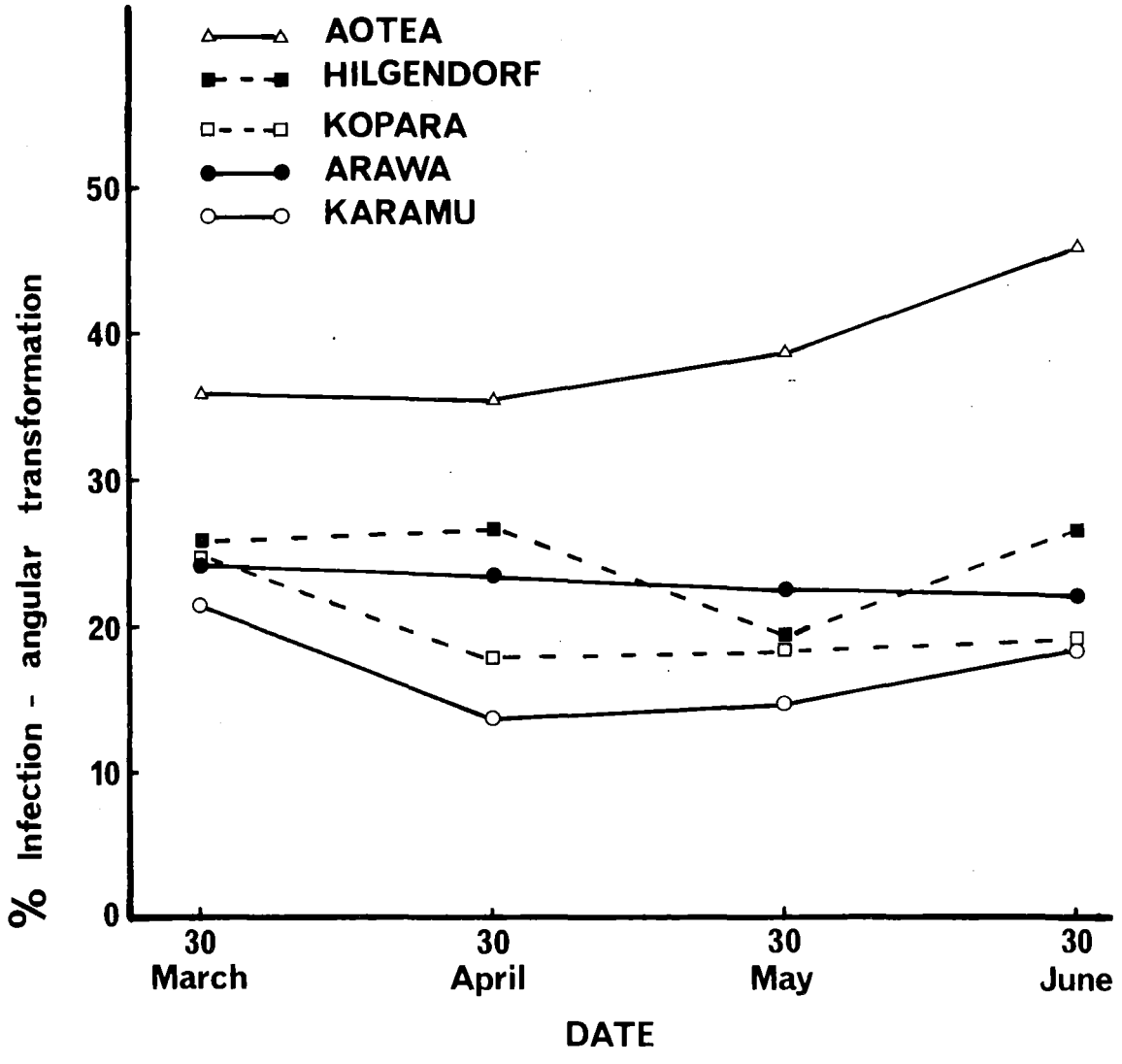


TABLE 10: Perithecia of *Mycosphaerella* sp. on bottom leaf in stubble of Lincoln College Variety Trial.

Wheat Variety	No. of perithecia per leaf (avg. 40 leaves)
Aotea	68.25 a*
Hilgendorf	41.37 b
Arawa	35.00 b
Kopara	33.25 b
Karamu	33.25 b

* Duncans multiple range test-non-significant groups 1% level.

6.34 Laboratory Trials

In the laboratory, varietal reactions of wheat to *S. tritici* and *S. nodorum* were studied, using a detached leaf technique. Leaf portions 4 cm in length from leaf 2 of 30 day old wheat plants grown in the glasshouse were inoculated with a spore suspension of 1×10^6 spores/ml by:

a) placing two drops (each 30 µl) of spore suspension on the adaxial surface of each leaf.

b) spraying the adaxial surface of the leaf till runoff.

After inoculation, the leaves were floated adaxial side uppermost on 10 mls of 50 ppm benzimidazole solution in a petri dish, two leaves from each plant on the same

dish. There were four dishes of each variety for each treatment, and a fifth set of dishes contained leaves treated with water. The dishes were placed at 22 ± 2°C under full light for the duration of the experiment.

After 16 days for *S. nodorum* and 21 days for *S. tritici*, leaves were assessed on the basis of percentage of photosynthetic area of the leaf affected. A scale from 0-5 was used, with 0 representing no infection, and 5 representing severe infection with *Septoria*.

6.35 Results

TABLE 11: Reaction of Wheat Varieties in Detached Leaf Culture to Inoculation with *Septoria* species.

Wheat Variety	*S. tritici* (21 days after inoc)		*S. nodorum* (16 days after inoc)	
	SPRAY a	DROP b	SPRAY	DROP
All water-inoculated leaves	0	0	0	0
Hilgendorf	1.2*	1.0	1.0	1.4
Kopara	1.5	0.5	1.5	1.0
Arawa	1.7	1.5	2.2	2.0
Aotea	2.8	2.2	2.4	2.5
Gamenya	3.0	2.4	5.0	2.7

a = inoculum ($1x10^6$ spores/ml) sprayed onto adaxial surface of leaf.

b = inoculum ($1x10^6$ spores/ml) placed on adaxial surface of leaf in two 30 µl drops.

* = scale representing degree of infection (0 = no infection; 5 = severe infection).

Baker (1970) stated that a high susceptibility to *S. nodorum* was associated with diffuse lesions and a large chlorotic area, while tolerance was associated with the limiting of lesion size rather than actual prevention of infection. With *S. tritici*, increased susceptibility was characterized by an increase in the number of lesions which eventually coalesced. A tolerant variety retained the green colour of the leaves, and lesions were limited in either number or size or both.

These definitions can be used to interpret the results from this experiment which showed that the varieties Aotea and Gamenya were particularly susceptible to both pathogens, while varieties Hilgendorf and Kopara showed signs of tolerance (Table 11; Plate 13). These results support the observations made in the field, and also show that the variety Karamu is more susceptible to *S. nodorum* than *S. tritici*. For the other varieties, the degree of susceptibility or tolerance was similar with both pathogens.

The chlorosis associated with susceptibility to the pathogens is suggestive of toxin production by fungi. Hilu and Bever (1957) demonstrated that *S. tritici* produced a metabolic by-product in culture which caused lesions on the host plant similar to those incited by the pathogen, but they did not isolate this substance, Jones (1974) believed that both *S. tritici* and *S. nodorum* produced a toxin which aided in the infection process. If this is the case, tolerance could well be associated with the ability of the plant to withstand the effects of this toxin, and thus restrict the lesions formed to a small area of the leaf.

Sanderson (1964b) found that variety Hilgendorf showed a degree of tolerance to *S. tritici*, and that Aotea was particularly susceptible. Ten years later, these two varieties react to *S. tritici* in the same way, as shown by both field and laboratory work. If the use of resistant varieties is ever undertaken as a means of controlling *Septoria* diseases in wheat in New Zealand, varieties Hilgendorf and Kopara could be used as a basis for resistance.

6.4 DISCUSSION

In the long term, the use of resistant varieties would seem to be the best method of control of *S. nodorum* and *S. tritici* in New Zealand wheat crops, and the required genetic material may already be present in some of our present varieties. However, a breeding programme to select for resistance would take some time to complete. In the meantime, the research as discussed in chapter 5, indicates that the practice of 'clean farming' would be an excellent control measure, by the removal of the sources of inoculum through burning or the deep ploughing of stubble, and elimination of volunteer plants.

Some measure of control can be achieved through chemical spraying of the wheat crop, but in New Zealand, the balance between increase in grain yield as against cost of treatment is marginal. Spraying should only be recommended in a season favourable to *Septoria* infection (moist and humid conditions), and applied at G.S. 9 to G.S. 10, to give protection to that part of the plant above the flag leaf node.

SECTION A 3: DISCUSSION

Both *Septoria tritici* and *Septoria nodorum* were isolated from wheat leaves from the field, the latter for the first time in New Zealand. *S. tritici* symptoms were common on leaves of wheat plants in the 1973 crop, and also on volunteer wheat plants in the stubble of the 1973 crop. *S. nodorum* symptoms were not detected in the 1973 crop, and were only found on the lower leaves of volunteer wheat plants in late winter/early spring of 1974. However, laboratory tests showed that infection of volunteer wheat plants by *S. nodorum* had occurred in the field in autumn of 1974, but symptoms were not produced. Experiments in the laboratory indicated that climatic conditions including humidity, temperature and light affected pycnidial formation by *S. nodorum*, i.e. under high humidity, NUV light and temperatures of 18-24^{o}C, pycnidia typical of *S. nodorum* were produced. It would seem that this fungus requires rather exacting conditions before symptom expression occurs, but further work is required to determine these conditions.

The presence of *Septoria nodorum* in wheat crops has now been reported from Canterbury, Otago (Arnst, 1975) and Southland (Sanderson, 1974). However, these reports have all been made within the last three years. If it is assumed that the fungus has been introduced into New Zealand fairly recently, then its spread must have been rapid, as it has

been recorded from the major South Island wheat growing areas. This suggests seed-borne inoculum as the spreading agent, but tests on random samples failed to confirm that it was seed-borne in New Zealand, and Matthews (1975) has also failed to detect seed-borne *S. nodorum* in routine tests at the Seed Testing Station, Palmerston North.

Overseas reports (Shipton, et al., 1971) suggest that where *S. tritici* is found, *S. nodorum* is also usually present. *S. tritici* has been recognised in New Zealand since 1949 (Wenham, 1959), but Wenham failed to detect *S. nodorum*. It is possible that the pathogen has been present in New Zealand wheat crops for many years, and that climatic conditions (e.g. hot dry summers), have been such that symptom expression does not usually occur, and pycnidia form only rarely. However, this seems unlikely in the view of recent reports of the presence of the disease.

There is also the possibility that wind-borne ascospores have been responsible for the rapid spread of the disease from an initial inoculum source. The sexual stages of *S. nodorum (Leptosphaeria nodorum)* and *S. tritici (Mycosphaerella* sp.) were found in wheat stubble throughout autumn and winter of 1974. These perithecia released wind-borne ascospores in response to moisture (either rain or dew) throughout this period. Sanderson (1972b) noted that these ascospores can be carried for several miles from the point of release, and it seems probable that infection in autumn-sown crops could be initiated in this way, especially

in those crops isolated from any other source of inoculum. Pycnidiospores could not initiate infection in this way as they are splash dispersed, and thus cannot move far from the pycnidium. Thus in New Zealand, ascospores could be an important primary inoculum source.

The presence of perithecia of both species in the stubble, and also the presence of pycnidia on volunteer wheat plants growing in the stubble suggests a practical control method for both pathogens. This is the practise of 'clean farming', i.e. burning or deep ploughing of stubble, and removal of volunteer plants. *S. tritici* is not seed-borne, and on present evidence, it seems that in New Zealand, neither is *S. nodorum*, hence stubble and volunteer plants appear to be the most important source of primary inoculum. The presence of either pathogen on alternate hosts has not been demonstrated in New Zealand. Careful control of this phase of the pathogens on wheat stubble could, therefore, reduce the incidence of either fungus.

Unfortunately, it does not seem possible to ensure that every farmer removes stubble or volunteer plants, and other control methods may have to be investigated. Foliar spraying for control of *S. tritici* can effect an increase in yield, but the economics of this operation are questionable. So far, *S. nodorum* has been difficult to find in wheat crops so the effects of this pathogen on yield cannot be determined. Unless the pathogen begins to severely attack the heads of wheat, fungicide spraying is not likely

to be economical (Jones, 1974). This leaves the use of resistant varieties as a control measure. Preliminary work showed that New Zealand already has varieties showing signs of tolerance to both pathogens, so that the genetic material needed for a breeding programme may already be present.

At present, *S. nodorum* and *S. tritici* do not appear to be major pathogens of wheat in New Zealand. *S. tritici* can reduce yields, through causing a reduction in the photosynthetic areas of the flag leaf which results in a reduction in grain weight, however the costs involved in foliar spraying to control this pathogen on the flag leaf may not be matched by the increase in grain yield. The presence of *S. nodorum* in New Zealand is perhaps more disturbing, as overseas, and especially in Great Britain, this fungus is a severe head pathogen. It appears that climatic conditions in New Zealand do not normally suit the fungus, and that it does not reach the head, but this is still to be determined. Both fungi have the potential to become major pathogens of the wheat crop, but control through the removal of the main primary inoculum source (stubble), could greatly reduce the chances of this occurring.

PART B. BUNT OF WHEAT

SECTION B 1: LITERATURE REVIEW

C H A P T E R VII

THE BUNT FUNGI

7.1 BIOLOGY OF THE BUNT FUNGI

7.11 Introduction

Bunt, or stinking smut of wheat is caused by two allied species of the lower Basidiomycetes, *Tilletia caries* (DC) Tul., and *Tilletia foetida* (Wallr.) Liro. Both species have been recorded in New Zealand by Cunningham (1924). Losses caused by bunt are due to the replacement of the grain by smut spores, the outer layers of the grain being left intact. For many years the disease has been controlled effectively by seed treatment and at present seems to be rare in New Zealand wheat crops.

7.12 Morphology

The two species differ chiefly in their spore morphology, which was first described in New Zealand by Cunningham (1924).

a) *Tilletia caries* - teliospores - globose to subglobose; 15-23 µm in diameter; spore wall is reticulated, with shallow meshes to deep reticulations; colour grey-brown to red-brown.

b) *Tilletia foetida* - teliospores - globose to elongate and tending to be more irregular in shape; 17-20 µm in diameter; spore wall smooth. Spore colour usually darker than that of *T. caries*, varying from grey-brown to dark olive-brown.

The hyphae of both species are septate, 2-3 µm thick, and have comparatively thick hyaline walls (Cunningham, 1924). The mycelium is generally difficult to detect in the host, but can generally be observed in the vicinity of the developing teliosori.

7.13 Symptoms of the disease

Until the heads of infected plants emerge from the 'boot' stage (G.S. 10.5), distinct symptoms are not always obvious. Stem length may be shortened and tillering increased (Butler and Jones, 1961; Agrios, 1970). After emergence, bunted heads take on a blue-green appearance, and are wider due to spreading of the glumes (Agrios, 1970; Sanderson, 1972b). Infected kernels have the entire grain contents replaced by a sooty, black powdery mass of fungal spores and the grains are plumper and shorter than the healthy grain. Bunt balls, when broken, emit a characteristic pungent odour, reminiscent of decaying fish, hence the name 'stinking smut'.

7.14 Disease Cycle

At harvest, or during storage of the grain, the bunt ball containing teliospores may be broken, and teliospores contaminate clean grain by adhering to the seed coat.

Germination of wheat seeds can occur during conditions which favour germination of teliospores, such as cool temperatures (6-12^{o}C) and low soil moisture following sowing (Agrios, 1970). When a teliospore germinates, a basidium is formed, from which 8-16 basidiospores or primary sporidia develop. These are long, hyaline and uninucleate. The primary sporidia then fuse in pairs, by forming lateral branches between compatible mating types, so that a binucleate, H-shaped structure is formed. Dikaryosis then occurs, through exchange of nuclei from each former primary sporidium. This structure then germinates and produces a short hypha on which dikaryotic secondary sporidia are formed. Germination of these secondary sporidia produces a dikaryotic mycelium.

This mycelium attacks the wheat seedling, penetrating it directly. After penetration, it grows intercellularly, through the stem, eventually reaching the meristem of the growing point. At this stage it causes little interference with the plant, but when the reproductive organs form, the mycelium invades the head. As the grain fills and matures, the mycelium replaces all the inner tissue (embryo and endosperm) except for the pericarp of the grain which remains as a cover for the fungal mass.

Once this stage is reached, the binucleate mycelium is transformed into thick-walled teliospores, leaving a smutted grain capable of infecting clean seed in the crop at harvest.

7.15 Factors that Affect Infection

Infection occurs over a soil temperature range of 5-20°C, but is greatest about 10°C (Butler and Jones, 1961). Below 5°C, spore germination and mycelial growth are poor. The optimum temperature for spore germination is below that which favours rapid growth of both winter and spring wheat varieties. It has been suggested that the seedling is only susceptible to infection up to time of emergence of the first green leaf from the coleoptile, so that the longer a plant is kept at a growth stage where it is susceptible, the greater will be the chance of successful infection (Butler and Jones, 1961).

Soil moisture is another important factor, as bunt is inhibited in very wet or very dry soils (Agrios, 1970). High moisture, while reducing rapid germination of the wheat, inhibits spore germination through lack of oxygen (Butler and Jones, 1961).

The bunt teliospores do not germinate in acid soils, (Butler and Jones, 1961) -pH 5 has been found to be the lower limit for germination so that in more acid soils, infection would not occur.

7.2 INCIDENCE OF BUNT IN NEW ZEALAND WHEAT CROPS

Bunt has probably been present in New Zealand wheat crops ever since wheat was first introduced. Early records of the Department of Agriculture (Annual Report, 1900 and 1901), show that smutting of wheat was prevalent, and Kirk (1906) reported that 'the smuts as usual were commonly occurring'. Neill (1923) noted that losses from 5-40% of the crop could be attributed to stinking smut where no preventive measures were used.

However by 1937, Blair (1937) could state that, 'it is possible that covered smut of wheat is .... of little importance throughout the wheat growing districts', because of the effects of seed treatment. Reports from seed merchants throughout the South Island showed that during the period 1932-1937, there were a number of infected lines, but the degree of infection was low. In many cases, infection was traced to absence of fungicide treatment of wheat seed.

Blair (1950) examined the 1947 and 1948 harvests, and found that the wheat seed samples carried *Tilletia* smut fungi to the extent of 17.4% and 8.9% over these two seasons. It was clear that foci of infection were in existance, and thus Blair (1956) recommended that preventive treatments be continued. Seed wheat disinfection has been a regular practice and during the period 1951-1958, stinking or covered smut was not seen in wheat crops (Smith and Blair, 1959).

However, Blair (1961) appraised the evidence for and against the continuation of seed wheat disinfection, because the disease had not been seen for a number of years, and the treatment was costing the farming community $40,000 per annum. Blair concluded that continuation of disinfection was not essential, but perhaps should be continued to ensure that bunt did not re-occur.

The need for continuation of seed treatment has been demonstrated a number of times in the past decade. Sanderson (1964a), Blair and Sanderson (1967), Close (1970) and Mulholland (1972) have all reported outbreaks of the disease in crops in the South Island. Some of those involved large acreages where wheat was so badly smutted it could not be used for milling purposes. In each case the farmer had sown un-treated seed. As long as disinfection of seed wheat is continued, bunt should never be a problem in New Zealand wheat crops.

7.3 CONTROL OF BUNT IN NEW ZEALAND

Although the practice was not common, chemical control of teliospores on seed wheat was used in New Zealand before the turn of the century. Ivey (1881) demonstrated that copper sulphate applied to the seed could control bunt. The seed was 'pickled' in a copper sulphate solution, but germination damage was common, especially if there was any time interval between treatment and sowing.

Until the 1920's, a liquid steep was the method employed for bunt control in wheat seed. A large number of chemicals were said to control the disease, but the Department of Agriculture recommended copper sulphate, formalin, potassium sulphide or mercuric chloride (Kirk, 1906). Neill (1926), in a series of experiments, tested all chemicals then available for the control of bunt, and showed that two methods of seed treatment were best under New Zealand conditions. These were a formalin steep, and the use of copper carbonate dusts. However the use of a steep considerably lessened seed viability, and a seed dusting was recommended.

A shortage of copper in Germany during the First World War lead to the introduction of organo-mercury dusts, and in New Zealand, commercial organo-mercuries such as Ceresan and Agrosan-G were soon in common use. Neill (1933) found them better than copper carbonate, which frequently failed to give complete disinfection at lower rates. Seed merchants installed dusting machinery, and farmers purchased seed wheat already dusted. Blair (1937) noted that 'it (dust treatment) is universally applied on seed wheat in this country'.

Although the organo-mercury dust treatment proved highly effective in controlling bunt, it had two main disadvantages. The most important of these was that the dusting procedure created a health hazard to men operating the dusting machinery, for the mercurial dust was poisonous. To try to overcome this, a slurry method was used but there

was still the danger of dust after the slurry had dried on the seed. The second disadvantage concerned germination of treated seed. Smith and Blair (1959) showed that application of organo-mercuric dusts to wheat seed with a moisture content above 15% reduced germination percentage.

In the 1950's, a number of non-mercurial disinfectants were marketed, and Smith and Blair (1959) found that a non-mercury fungicide containing thiram was less effective in bunt control, but did not adversely affect germination, even when high moisture seed was to be treated. Because of this, Allen (1961) recommended that a mixture of a thiram containing fungicide, and a mercurial fungicide be used. Sanderson (1964a) found that thiram, captan and the oxyquinones gave control equal to mercury, and without the risk of pre-emergence damage as sometimes occurred under cold, wet conditions.

Infection of wheat by *Tilletia* sp. can occur either by direct penetration of the seed from spores adhering to the seed coat, or by soil-borne spores infecting the coleoptile as it emerges. Coating the seed with an effective fungicide will control seed-borne inoculum but may not give control of soil-borne inoculum. Mulholland (1972), investigated the use of systemic fungicides of the carboxin and benzimidazole types for control of the soil-borne inoculum, as it was considered that movement of the active material to the growing plants might protect the seedling coleoptile.

However, results of trials in the 1970-1971 season did not support this, as the level of infection was considerably greater in infected soil than in clean soil when systemic materials were used.

At present captan is the fungicide in use by most seed merchants for the control of bunt, but there are a number of other fungicides which could be used equally effectively. Chemical control of bunt has been so effective that no alternate methods have ever been required. Breeding resistant wheat varieties was attempted in England (Butler and Jones, 1961) but not in New Zealand.

7.4 SEED TREATMENT AND GERMINATION

Trials to assess the effect of disinfection on the germination of seed wheat in New Zealand have been numerous, and have often produced very variable results. Neill (1927) found that liquid disinfections (formalin, copper sulphate), reduced germination while organo-mercury compounds as dusts had no appreciable effect. Neill (1936) later showed that organo-mercury dusts increased plant establishment by 14-15%, but reduced field emergence in other experiments (Neill, 1938). In an attempt to explain this discrepancy, the soil conditions in which the germination was occurring were studied. Blair (1938) found that in ordinary field soil under optimum conditions for germination, disinfection produced no more than a 1% increase in plant establishment.

However, in cold, wet conditions, when germination would be slow, disinfection treatments gave a 10% improvement in germination, mainly because disinfection controlled rot-inducing fungi such as *Fusarium* and *Pythium*.

After Blair (1950) showed that the amount of dust on seed was often inadequate and poorly applied, there was a tendency for dosage to be increased (Blair, 1961), and this corresponded with increasing reports of poor germination. Blair proposed the hypothesis that the use of header harvesters rather than binding and threshing, was leading to increasing amounts of wheat being disinfected at too high a moisture level. Smith and Blair (1959) showed that when organo-mercury dust was applied to seed of moisture content above 15%, curled and stunted seedlings and greatly reduced field emergence resulted. This work also showed that the damaging effects did not occur with some non-mercury fungicides, even where wheat of a moisture content greater than 15% was treated. Sanderson (1964a) compared an organo-mercury with non-mercurial fungicides. At the recommended application rates, there was no significant reduction in germination with either type, but when application rates were doubled, germination of the organo-mercury treated seed was less than 10%.

In the light of these results, Blair and Sanderson (1967) recommended the use of non-mercurial seed dressings for control of bunt so that crop failures due to germination injury could be avoided.

C H A P T E R VIII

ASSESSMENT OF FUNGICIDAL ACTIVITY BY BIOASSAY

8.1 INTRODUCTION

Chemicals for crop and seed protection must perform well under practical conditions, and field trials are essential (Martin, 1964). However field trials are expensive, laborious and lengthy procedures in which to compare the effectiveness of a range of chemicals. In addition, there are a large number of variables, which often are not able to be controlled.

In order to avoid some of these problems, and to obtain a quick and repeatable test, laboratory bioassay methods can be used for evaluation of fungicidal activity. The primary object of the bioassay is to determine the response of the individual organism exposed to the toxicant under conditions which are repeatable and in which only one of the factors can be altered (Martin, 1964). The factors that are varied are usually the amount of toxicant to which the organism is exposed, and the time of exposure, although other variations may arise through differences in resistance of the individual organisms in their exposure to the toxicant, and the environmental conditions.

Many methods have been used in the laboratory to detect the activity of fungicides. However there is no single mode of action on which to base an assay of fungitoxity, and so

criteria for toxic action are described in general terms as inhibition of growth and development (Lukens, 1971). Three measurable criteria are growth, spore germination and respiration. In vitro, these criteria may be used in testing the activity of fungicides (Horsfall, 1956; McCallan, Hamilton and Mills, 1959; Lukens, 1971). The activity is measured in terms of the response of a treated culture to an untreated one.

8.2 EFFECTS ON GROWTH

8.21 Direct Methods

Direct methods of testing involve measuring the reaction of microbial cells to chemicals to show either effects on individuals or changes in cell numbers. For fungi as test organisms, there are two main methods:

a) Colony Diameter.

Colony diameter measurements have been widely used in evaluating the effects of fungicides (Horsfall, 1956). In general test chemicals have been incorporated into agar medium which was inoculated later with indicator organisms. The rate of increase of colony diameter, or the colony diameter after a constant incubation period, may be measured, and differences in growth between organisms on treated media and those untreated can be established.

b) Dry Weight of Cells.

Measurement of dry weight of cells growing on fungicide treated media has been used in plant pathology. The mycelium is harvested, dried and then weighed, but the inherent errors of dry weight determinations (Mallette, 1969; Lukens, 1971) make this method of limited value for testing fungicidal activity.

8.22 Agar Diffusion Techniques

In the study of various types of toxicants, agar diffusion techniques employing micro-organisms as indicators have been used. In these methods, antifungal chemicals, placed in a reservoir, diffuse out through a solid medium which has been inoculated with the test organism. If the compounds under test are fungistatic or fungicidal to the indicator organism, a zone of inhibition of growth is seen, after incubation, around the reservoir. The effects of test compounds on the organism are measured by the size of the inhibition zone produced.

Two methods of inoculation of solid media with test organisms have been used:

a) by spreading an inoculum over the surface of previously solidified media,

b) by adding inoculum to sterile liquified agar media and then allowing it to solidify.

The second approach has been more commonly used, because it allows more accurate control of the test inoculum applied (Falloon, 1972).

There have been many different types of chemical reservoir used. Falloon, (1972), reviewed the types used in antibiotic assays. In the field of plant pathology, Palmiter and Keitt (1937) used small agar cylinders containing known amounts of fungicide, which were placed on the seeded plate. Depressions, or wells, in the agar, into which test chemicals were placed, have also been used (Leben and Keitt, 1950).

Absorbent paper discs have been widely used as chemical reservoirs, particularly in recent years in antibiotic sensitivity testing (Falloon, 1972). Thornberry (1950) and Leben and Keitt (1950) introduced the method to plant pathology.

A parallel development to the effects of toxicants on indicator organisms by the paper disc method, was the use of this agar diffusion method in plant pathology for determining the amount of fungicide on seeds. Mead (1945) and Machacek (1950) used treated seed as a reservoir, by placing it on the surface of seeded agar. Arny (1952) improved the diffusion into the agar by pushing the seed down into molten agar so that it was completely covered. The agar diffusion technique is used for the microbiological assay of fungicide-treated seeds by the Association of Official Seed Analysts (Kulik and Crosier, 1964). More recently, the agar diffusion technique has been used for determination of the amount of systemic fungicides in plant tissues (Erwin et al., 1971).

Factors that may cause variations and errors in agar diffusion methods have been critically studied, the most recent being that of Davis and Stout (1971a). Factors considered to be important were; agar characteristics, including agar depth, composition, pH and water content; inoculum concentration; incubation temperature; preparation and application of test chemicals. Davis and Stout (1971b) described a procedure to overcome or allow for variables occurring in diffusion methods.

The use of agar diffusion techniques for detecting dressings on treated seeds has been recently reviewed by Ehle (1973). He concluded that although sophisticated chemical and physical methods are available, the agar diffusion bioassay is of great value in checking seed treatments and can yield reliable information on the amount of dressing on seeds. It has the added advantages that it is simple and relatively inexpensive to perform, and a greater number of individual seeds per sample can be examined than would be feasible using physical or chemical methods.

8.3 METABOLIC RESPONSE METHODS

These methods measure the effects of test chemicals on metabolic functions of indicator organisms, and by comparison with untreated organisms, the sensitivities of test fungi are determined. They have been used particularly in analytical microbiology (Heatley, 1949).

8.31 Assessment of germination

The germination of fungus spores in the presence of a chemical has been particularly favoured by plant pathologists for assessing fungistatic and protectant action (McCallan and Wellman, 1942). As most fungicides are designed to prevent establishment of the pathogen, a measure of the effect of the fungicide on spores is important.

Slide germination tests are a standard method for evaluating protective fungicides (American Phytopathological Society, 1943), and for primary screening of fungicides. A deposition base, other than a glass microscope slide, for evaluating fungicides has been used; for example, filter paper discs and cellophane discs (Neely and Himelick, 1966).

8.32 Respiratory response

Inhibition of respiratory activity, as measured by oxygen uptake or CO_2 evolution, may be used in the assessment of fungicidal activity (Horsfall, 1956). However, there have been inaccuracies involved, and Lukens (1971) considered that this was because gas exchange is only a part of growth, and that not all the energy for growth comes from gas exchange.

8.4 DISCUSSION

Bunt is controlled by treating seed with fungicides, and it was necessary to select a suitable technique for measuring the amount of fungicide on seed to see if all seed was receiving an adequate dosage. The method of bioassay was chosen, the literature reviewed, and a method based on agar diffusion techniques was used in subsequent experiments (Chapter 10).

SECTION B 2: EXPERIMENTAL

C H A P T E R IX

INCIDENCE OF BUNT IN NEW ZEALAND WHEAT CROPS

9.1 INTRODUCTION

Although a problem in the early part of this century, the incidence of bunt in New Zealand crops over recent years has been generally low (Blair, 1950; Smith and Blair, 1959). However there have been isolated outbreaks within the last decade (Sanderson, 1964a; Close, 1970), and in one case contamination of the grain was such that the harvested grain was rejected for milling purposes (Mulholland, 1972). An investigation of these outbreaks proved that, in each case, untreated seed wheat had been sown, often for a number of years in succession (Blair and Sanderson, 1967; Mulholland, 1972).

The last survey of the incidence of bunt in New Zealand wheat crops was that by Blair (1950) who found that 17.4% and 8.9% respectively of the samples of the 1948 and 1949 harvests were contaminated with bunt spores. The recent outbreaks of bunt indicate that there are still pockets of infection throughout the country. Therefore, samples of the 1972-73 harvest were examined to determine the incidence and distribution of bunt in New Zealand.

9.2 SAMPLING METHOD

Samples of wheat grain submitted to the Wheat Research Institute laboratory were used in this study. As 15,000 samples are received by the laboratory each season (Merideth, 1970), two methods of sampling had to be adopted.

a) The Wheat Research Institute, at the end of each testing season, forms a varietal bulk from the remainders of all the officially drawn wheat samples sent to it during the season. It was therefore possible to draw from these varietal bulks, a sample (one kilogram) representative of the variety for that particular year. This was done (where available) for the varieties Aotea, Arawa, Hilgendorf, Triple Dirk, Gamenya, Raven, Karamu and Kopara, for the years 1970-73. These were tested by the method described in section 9.3, to determine whether teliospores of bunt were present and whether any one variety contained significantly more *Tilletia* teliospores than the others (Table 12). These tests showed that for each of the years 1970-1973, the variety Aotea had significantly higher levels of *Tilletia* teliospores. (Appendix 3). Therefore, samples of this variety, from the 1973 harvest, were the only ones to be tested.

b) In order to select samples from different areas of New Zealand, the area of Aotea grown in the different counties in New Zealand was determined from data supplied by the Government Statistician for 1969-1970 (Copp, 1970). For every 200 ha of Aotea wheat grown in a county, one sample (250g) was picked at random from all the samples of Aotea wheat from that county sent in to the Wheat Research Institute, Christchurch.

TABLE 12: Occurrance of *Tilletia* teliospores on bulk grain samples of different wheat varieties from 1970-1973 harvests.

Variety	No. samples containing teliospores out of 10 reps. tested per bulk sample				
	1970	1971	1972	1973	Total of 4 seasons
Aotea	4t.m.[2]	2t.m.	4t.m.	1t.m.	11t.m.
Arawa	1t.m.	0	1t.m.	0	2t.m.
Hilgendorf	0	1t.m.	0	0	1t.m.
Raven	0	0	1t.m.	-3	1t.m.
Triple Dirk	1t.m.	1t.m.	0	0	2t.m.
Gamenya	-	1t.m.	0	1t.m.	2t.m.
Kopara	-	0	0	0	0
Karamu	-	-	-	0	0

1 - after the slides of each sample were prepared, 25 microscope fields (x320) were examined at random for teliospores.

2 - the following load ratings of Russell and Ledingham (1941) were used:

Trace minus (t.m.) = mean of less than 1 teliospore/microscope field*

Trace (t.) = " " 1 teliospore/microscope field

Trace plus (t.p.) = " " 2-3 " " "

Slight (sl.) = " " 4-10 " " "

Moderate (m.) = " " 10-50 " " "

Severe (sv.) = " " >50 " " "

3 - not tested.

* Field diameter at x320 = 20 µm.

9.3 METHOD OF TESTING

The method used for testing wheat for the presence of *Tilletia* teliospores has been the centrifuge method, suggested by Simmonds and Mead (1933), and used extensively by Russell and Ledingham (1941) and Blair (1950). In their studies, 40 g of grain was mixed with 60 ml of sterile water and shaken mechanically for 5 minutes, after which a 10 ml aliquot of the suspension was centrifuged at 2,500 r.p.m. for 5 minutes. Most of the supernatant was discarded but 0.5 ml in the base of the tube was either smeared onto a microscope slide and dried, or a drop of it on a standard wire loop was placed on a glass slide and covered with a cover glass.

Cherewick (1944) has discussed the limitations to this method, important points being that centrifuging does not completely sediment all of the spores, and that one washing with distilled water may not be enough to remove all the spores from the seed. Cherewick points out, however, that a drop of a proprietary wetting agent may overcome these difficulties, and Wallen and Ednie (1972) used 0.1% Tween 20 in their washing solution.

However, the centrifuge method is rather laborious, and in an effort to find more rapid techniques of equal or improved accuracy, a method suggested by Close (1973) was tried. This involved the use of a 0.45 µm cellulose membrane of 25 mm diameter (Hawg 02500, Millipore Corporation, U.S.A.).

From the samples, 20 g of grain were weighed and placed in a 500 ml Erlenmeyer flask. To this, 30 cc of distilled water plus 0.1% Tween 20 was added, and the mixture agitated for 5 minutes on a magnetic stirrer. This was then filtered through cheese cloth, and 10 cc of the solution drawn into a syringe. A cellulose membrane was placed in position in the "Millipore" holder, the syringe attached to the holder, and the liquid passed through the membrane by depressing the plunger on the syringe. When the syringe was empty, the membrane was removed from the holder and placed on a microscope slide. A drop of clear lactophenol was placed on the membrane to disperse any aggregated particles, and a cover slip placed on the membrane. This was then examined with a microscope at x320 for the presence of *Tilletia* teliospores. Care was taken to distinguish between teliospores of *T. caries*, and those of *T. foetida*, the major distinguishing characteristic being the spore wall - reticulated for *T. caries* and smooth for *T. foetida*. *T. foetida* teliospores also tend to be irregular in shape, often elongated, and generally darker in colour than those of *T. caries* (Cunningham, 1924).

9.4 PROCEDURE ADOPTED

9.41 Centrifuge versus Cellulose Membrane

To test whether there were any major differences between the two methods, samples of clean wheat grains were artificially inoculated in the laboratory. Bunt balls

containing *T. caries* teliospores were added to weighed lots of clean grain to give a concentration of 0.1% weight for weight. The grain was in conical flasks. Bunt balls were crushed and the stoppered flasks shaken for 5 minutes, to mix the teliospores thoroughly with the grain.

This artificially inoculated grain was then tested by both procedures, and slides from each examined. Both methods produced moderate load ratings, based on the ratings of Russell and Ledingham (1941). There was no significant difference between the two methods at the 1% level (Appendix 3), although the cellulose membrane method seemed to trap more spores. On the basis of this evidence it was decided to use the more convenient cellulose membrane method.

9.42 Number of Washings

The artificially inoculated grain was used to determine the number of washings in distilled water needed to remove the spores from the grain. The same samples of grain were tested 3 times by the cellulose membrane method, and the number of spores in 25 fields counted. Ten different grain samples were used, and each time, the bulk of the spores were removed in the first washing, with only a very few left to be removed by the second washing, and none at all by the third washing (Table 13).

TABLE 13: Effect of number of washings on removal of *Tilletia caries* teliospores from artificially inoculated Aotea wheat grains.

Sample	Number of spores counted in 25 microscope fields (x320)		
	1st Washing	2nd Washing	3rd Washing
1	246	10	0
2	310	8	0
3	288	1	0
4	202	3	0
5	492	0	0
6	427	0	0
7	349	0	0
8	516	1	0
9	389	17	0
10	222	10	0

Similar results were obtained with naturally infected samples of Aotea wheat (Table 14). For the remainder of the testing, only one wash in distilled water + 0.1% Tween 20 was used.

TABLE 14: Effect of number of washings on removal of *Tilletia caries* teliospores from naturally infected Aotea wheat grain.

Sample	Number of spores counted in 25 microscope fields (x320)		
	1st Washing	2nd Washing	3rd Washing
1	4	0	0
2	5	0	0
3	2	0	0
4	6	1	0
5	7	0	0
6	5	0	0

9.43 Number of fields examined

Cherewick (1944) recommended that 8 microscope fields should be counted when smut spores were numerous, and more when only a few spores were present. Blair (1950) counted 30 fields per sample, using oil immersion magnification. However, with the filter paper discs, oil immersion was not necessary as the spores could be seen quite clearly against the white background of the filter paper under a magnification of x320.

In a preliminary experiment, the number of fields that needed to be examined to give an accurate assessment of the number of spores on the whole cellulose membrane was determined. Spores from 6 samples of wheat were trapped on the membrane

and placed on a microscope slide in the manner described. For each sample, the number of spores in 10, 20, 30, 40 and 50 fields were counted. Table 15 shows that the total for 50 fields were twice the totals for 30 fields, so that accuracy would not be lost by counting fewer than 50 fields. Accordingly, all results shown are for 25 microscope fields per sample.

TABLE 15: Number of *T. caries* teliospores found in samples from naturally infected Aotea wheat grain, a range of 10-50 microscope fields being examined per field.

No. of fields	Number of teliospores per sample						Total
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
10f	2	1	1	2	2	1	9
20f	3	6	2	6	3	2	22
30f	5	9	5	6	3	5	33
40f	9	10	10	10	5	11	55
50f	13	12	12	10	7	12	66

9.5 RESULTS

The results of observations on the occurence of bunt teliospores on harvested Aotea wheat for the 1972/73 season are in Table 16. All teliospores were those of *T. caries*, and *T. foetida* was not detected. The presence of teliospores in varietal bulks (Table 12), although low in all cases (<1 spore/

microscope field), suggested that there was still infection in crops throughout the country. When samples of the variety Aotea were tested, samples yielding spores were obtained from many of the wheat growing areas of New Zealand.

The results in Table 16 support the belief that bunt in wheat is at present reasonably controlled by seed disinfection. In addition, the results show that there has been little change in the situation since Blair's 1950 survey, when an incidence of bunt infection in 1947 of 17.4% and in 1948 of 8.9% of the samples was found (Blair, 1950). Blair also reported that the incidence of *T. foetida* in New Zealand wheat crops was very low. There was a 9% bunt infection in the samples examined from the 1973 harvest, but in all cases the teliospores occurred only as a trace or less. These were all teliospores of *T. caries*, as *T. foetida* was not detected.

It is known that a direct relationship exist between *Tilletia* teliospore load and the amount of bunt appearing in crops sown with infected seed. Heald (1921) concluded that maximum infection arose from spore loads between 3,500 and 15,000 per grain (200-1000 per microscope field). Holton and Heald (1941) noted that only very light infection would result when wheat seed carried no more than 500 spores per grain (30 per microscope field). Thus it is clear that contaminated samples detected in this survey were not in a category which would result in a severe outbreak of the disease. However, it is apparent that small foci of light infection continue to exist so that, if seed is not treated,

TABLE 16: Occurrence of *Tilletia* teliospores on Aotea wheat grain from the 1973 harvest (total teliospores in 25 microscope fields (x320) per sample).

Sample origin (County)	No. of samples tested	No. of samples containing teliospores[1]
North Island		
Hawkes Bay	1	1 trace minus[2]
Waipawa	3	0
Dannevirke	2	0
Waitotara	2	0
Wanganui	2	0
Rangitikei	6	1 trace minus
Oroua	1	0
Manawatu	2	0
Kairanga	1	0
Masterton	5	0
Featherston	4	0
South Island		
Marlborough	1	0
Awatere	1	0
Kaikoura	1	0
Amuri	6	1 trace minus
Cheviot	2	1 trace minus
Waipara	8	0
Ashley	4	0
Rangiora	5	1 trace 1 trace minus
Eyre	6	2 trace minus
Oxford	2	0
Malvern	10	0
Paparua	4	0
Waimairi	1	0
Ellesmere	20	1 trace minus
Ashburton	30	1 trace 1 trace minus
Wairewa	1	0
Geraldine	20	1 trace minus
Levels	10	3 trace minus
Mackenzie	5	1 trace minus
Waimate	10	0
Waitaki	20	2 trace minus
Waihemo	1	0
Taieri	4	0
Bruce	4	0
Clutha	4	0
Tuapeka	2	0
Lake	3	0
Vincent	4	1 trace minus
Southland	20	1 trace minus
Wallace	11	2 trace minus
TOTAL	244	2 trace 20 trace minus

1 = all *T. caries*, as no *T. foetida* detected.
2 = after spore load ratings of Russell and Ledingham (1941) - see Table 12.

severe infections could develop. It is obvious that treatment of wheat seed must continue, and every grain sown in New Zealand must be effectively treated so that the aim of complete control of this disease may be achieved.

C H A P T E R X

EFFICIENCY OF COMMERCIAL SEED WHEAT DISINFECTION

10.1 INTRODUCTION

Chemicals have been applied to wheat seed for control of bunt for over a century, although the methods of application and types of chemicals have changed considerably. The aim of seed treatment is to ensure that sufficient fungicidal material is present on each seed to provide the desired level of protection against bunt and other seed and soil-borne pathogens. It is important to ensure that the quantity on each seed is not likely to cause damage to seed or seedlings, or add appreciably to the cost of seed. However, these objectives are not always easy to achieve, as the quantity of fungicide required per seed is small, and an efficient coverage is dependent on the method of application.

Blair (1950), in an examination of the efficiency of seed-treatment machinery, measured the dust load on wheat seed, and found that only 43% of the treated samples were carrying the amount of dust (1½-2 ozs/bu.) recommended for the control of bunt. As a result, dusting efficiency was improved, through the introduction of new dusting machinery or the modification of existing types, so that up to 80% or more of the samples tested were thoroughly treated (Blair, 1960).

However, seed treatments for control of bunt changed from dusting with organo-mercurials to slurry treating with non-mercurial fungicides such as captan. Because there was no information available in New Zealand, the efficiency of seed wheat disinfection by slurry application was assessed by examining and testing seed from samples submitted by Canterbury seed merchants.

10.2 DIRECT EXAMINATION

Samples of slurry-treated seed wheat were received from 5 Canterbury seed merchants. All lines had been treated with captan at recommended rates by a slurry treater hereafter referred to as type A. The formulation Orthocide 75 (75% a.i. captan) was used in all cases and as it contained a red dye, it was possible to detect variations in the amount of dye on the seeds by visual examination (Plate 14). It would be valid to assume that the amount of red dye on each seed would be similar to the amount of captan on each seed.

From visual observations, 3 grades were defined as follows:

a) Nil to poor - from 0-20% coverage * of each seed

b) Medium - from 30-70% coverage of each seed

c) Heavy - from 80-100% coverage of each seed

* as shown by the red dye.

Two hundred seeds from each line were examined under a binocular microscope for the presence of the red captan slurry, and rated according to the grades defined. In all, 21 different lines of machine dressed and treated wheat seed were examined.

Results.

Table 17 shows that in any line of seed wheat, slurry-treated with captan, coverage by the fungicide was extremely variable, with only 50% of seeds examined falling into the medium grade. It was decided to confirm these observations by assessing the uniformity of wheat seed treatment, to determine how many seeds were carrying the correct fungicide load.

10.3 DETERMINATION OF UNIFORMITY OF SEED TREATMENT

10.31 Introduction

To determine the amount of fungicide on individual seeds, colorimetric chemical procedures have been used (Simon and Kulik, 1971), as they may be less variable than bioassay methods and more accurate. However, they are, in general, more expensive in that they often require skilled personnel as well as special equipment (Ehle, 1973).

The bioassay, on the other hand, has become a routine method for detecting and determining amounts of dressings on a variety of seeds (Ehle, 1973). It has the advantage of

TABLE 17: Percent of wheat seeds within treated lines of a number of cultivars, grouped according to fungicide coverage on visual assessment

Seed Merchant	Cultivars and lines of seed	Coverage Group		
		Nil to poor	Medium	Heavy
1	Kopara a	30	44	26
	" b	25	57	18
	" c	28	44	28
	Aotea a	27	53	20
	" b	27	50	23
	Hilgendorf a	35	39	26
	" b	27	61	12
	" c	32	44	24
	" d	32	48	20
	Karamu a	38	45	17
	" b	30	57	13
	" c	17	56	27
	Gamenya	28	60	12
	Raven	40	46	14
2	Arawa	46	47	7
3	Aotea	28	53	19
4	Aotea a	21	64	15
	" b	22	60	18
5	Kopara a	14	68	18
	" b	22	64	14
	Hilgendorf	15	63	22
	Range	15-46	39-68	7-28
	Mean	28	54	18

being simple and inexpensive while giving consistent results. As well, a greater number of seeds from a sample can be individually examined than would be feasible using physical or chemical methods.

In this work, information was required on both the rate and uniformity of fungicide treatment of wheat seed, rather than information as to the actual quantity of compound per unit seed surface. After assessing the evidence of a number of authors (Kulik and Crosier, 1964; Simon and Kulik, 1971; Ehle, 1973) it was considered that the bioassay would provide the information required faster and without much loss in accuracy and enable a greater number of samples to be examined. Accordingly, a bioassay, after the methods of Kulik and Crosier (1964), and Mitchell (1967) was developed.

10.32 Procedure

a) Theory

A drop of fungicide placed on the surface of solidified agar will diffuse through the agar. The growth of many micro-organisms is inhibited by fungicides. Therefore, if a population of a susceptible micro-organism is added to the agar, a drop of the fungicide on the agar will restrict the growth of the micro-organism to an area outside the lethal area of diffusion of the fungicide. The size of the inhibition zone will be related to the concentration of the chemical present.

b) Test organisms and inoculated media

Two fungi, *Glomerella cingulata* (Stonem.) Spauld and Schrenk, and *Aspergillus niger* van Teigh, were used as the test organisms. These were grown on PDA at 24°C in the dark until sporulating profusely, when 10 ml of sterile water was added to each plate and a spore suspension prepared. This suspension was diluted to approximately 1 x 10^5 spores/ml before being added to sterile culture media (PDA) which had been kept liquid at 48°C in a water bath. Chloramphenicol was then added to each litre of media to give a final concentration of 0.005 ppm, to inhibit the growth of any bacterial contaminants that may have been introduced. Ten ml of a 2,4-Dichlorophenoxyacetic acid solution (200 ppm) was added to each litre of media, to prevent germination of the wheat seeds, as this could interfere with the development of the inhibition zones.

c) Preparation of Plates

Twenty ml aliquots of inoculated media were aseptically pipetted in to sterile 9 cm disposable plastic petri dishes, which were gently rotated on a level bench to ensure a flat and uniform agar layer. The dishes were uniform in size and more suitable than glass because they reduced variation in agar thickness which has been shown to cause variation in tests (Davis and Stout, 1971a; 1971b).

Before the agar layer had set, and by use of a marked template, 5 seeds were pressed to the bottom of each plate, so that each seed was equidistant from the others,

and the entire surface of each seed was in contact with the agar. On cooling, the plates were incubated at 24°C in the dark for 96 hours. The diameter of the inhibition zone around each seed was then measured twice with vernier calipers, and the mean diameter determined.

d) Preparation of a standard curve

A standard curve was prepared in order to correlate the diameter of the zone of inhibition to the concentration of chemical present. Aotea wheat seeds were treated with captan slurry at the rates of ¼N, ½N, N, 2N and 4N (N= 58.5 g captan/100 kg seed - the recommended rate) by agitation of seed with the slurry on a Griffen flask shaker. After treatment, the seeds were allowed to dry, placed within the inoculated media, incubated at 24°C for 96 hours, and their zones of inhibition measured. One hundred seeds of each treatment rate were tested.

In using seeds to obtain a standard curve, it was assumed that most of the captan on the surface of the seed had diffused into the agar, and that each seed was covered with the same amount of slurry as all the others in its rate grouping. To check if this occurred, another method was tried, using filter paper discs.

The amount of captan slurry that would be applied to one seed at the recommended rate (N) was calculated as follows:

Avg. 1000 grain weight Aotea wheat = 35 g

$\therefore$ avg. weight one seed = 0.035 g

Recommended rate of captan application = 58.5 g/100 kg seed

$$\text{Amount captan on one seed} = \frac{58.5 \times 0.035}{100,000}$$

$$= 20 \text{ µg}$$

A slurry was prepared containing 1 g captan/litre solution, and 20 µl of this applied to each seed would produce 20 µg captan per seed.

Twenty ml aliquots of inoculated media were pipetted into plastic petri dishes in the manner already described. When cool, three absorbent paper discs were placed at equi-distant points on the agar surface with a needle. The paper discs had been cut from Whatman thick seed test paper with a paper punch, and were 6.2 mm in diameter. Twenty µl of each of the five slurry treatments (¼N, ½N, N, 2N, 4N) were applied to the discs using a 100 µl tuberculin syringe. These plates were then incubated at 24°C in the dark for 96 hours. Ten plates of each slurry concentration were tested.

e) Testing merchant treated samples

A random selection of 50 seeds from each line sent in by Canterbury seed merchants was tested in the manner described. All these lines had been treated by machine type A.

Seed treated by another slurry treater (type B) was also obtained, tested in the same manner, and the results compared with those of samples from the type A machine.

10.4 RESULTS

10.41 Standard Treatments

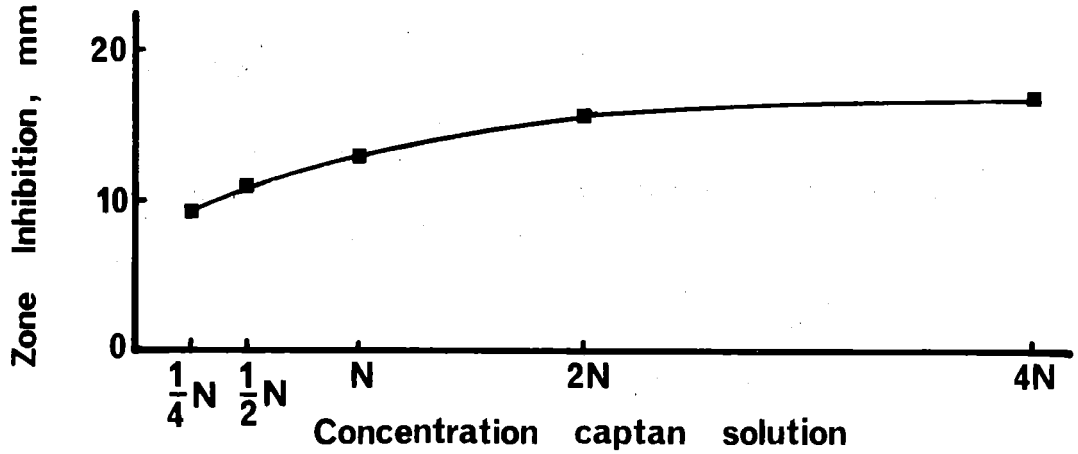
Results using *Aspergillus niger* as the test organism are presented in Table 18, figures 15 and 16, and plates 15 and 16. As similar results were obtained using *G. cingulata* as the test organism, only *A. niger* was used for subsequent experiments. There was little difference in size of zones of inhibition between the filter paper discs and seed method. Thus individual seeds appear to have received the correct amount of captan slurry.

10.42 Merchant Treated Samples

Figure 17, Table 19, and plate 17, all show that the coverage of commercially treated wheat seed with captan was extremely variable, as indicated by the great range of inhibition zones around the seeds tested. The results also show a marked difference in coverage between seed treated by machine type A, and that treated by machine type B. Figure 17 shows that nearly 30% of seed treated by machine type A had less than half the recommended (N) rate of captan per seed while 6% had virtually no captan on the seed at all. Table 19 shows that seed treated by machine type A ranged in coverage from no captan to over twice the recommended rate of captan per seed, while the average coverage was below the recommended rate. Seeds treated by machine type B were more uniformly treated (Figure 17; Table 19), although the amount of coverage did range from half to twice the recommended

FIG. 15.

a) Mean diameter of inhibition zone of *A. niger* by captan applied to filter paper discs at rates of ¼N, ½N, N, 2N, 4N.



b) Mean diameter of inhibition zones of *A. niger* by captan applied to Aotea wheat seeds at rates of ¼N, ½N, N, 2N, 4N.

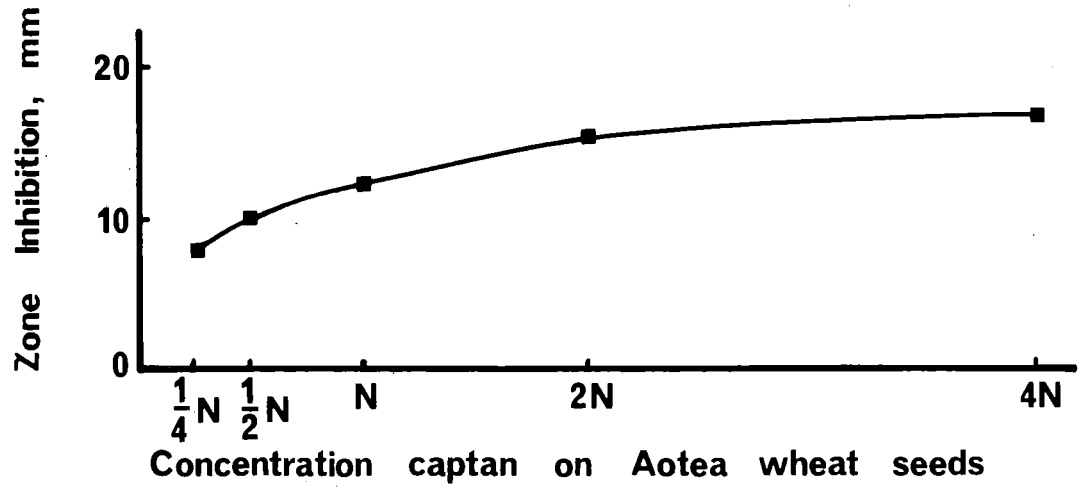


FIG. 16. Distribution of inhibition zone diameters of *A. niger* by captan applied to Aotea wheat seeds in the laboratory at standard rates of ¼N, ½N, N, 2N, 4N.

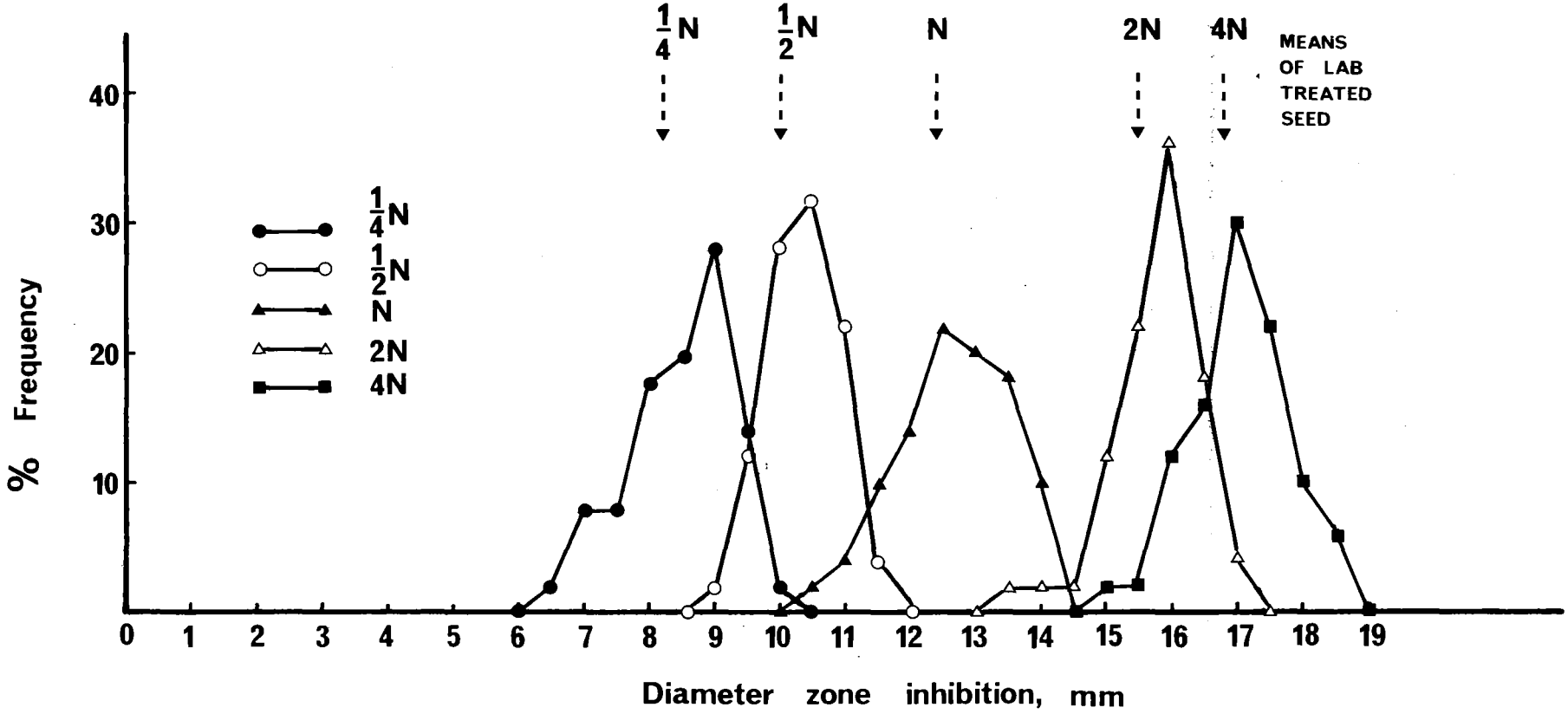
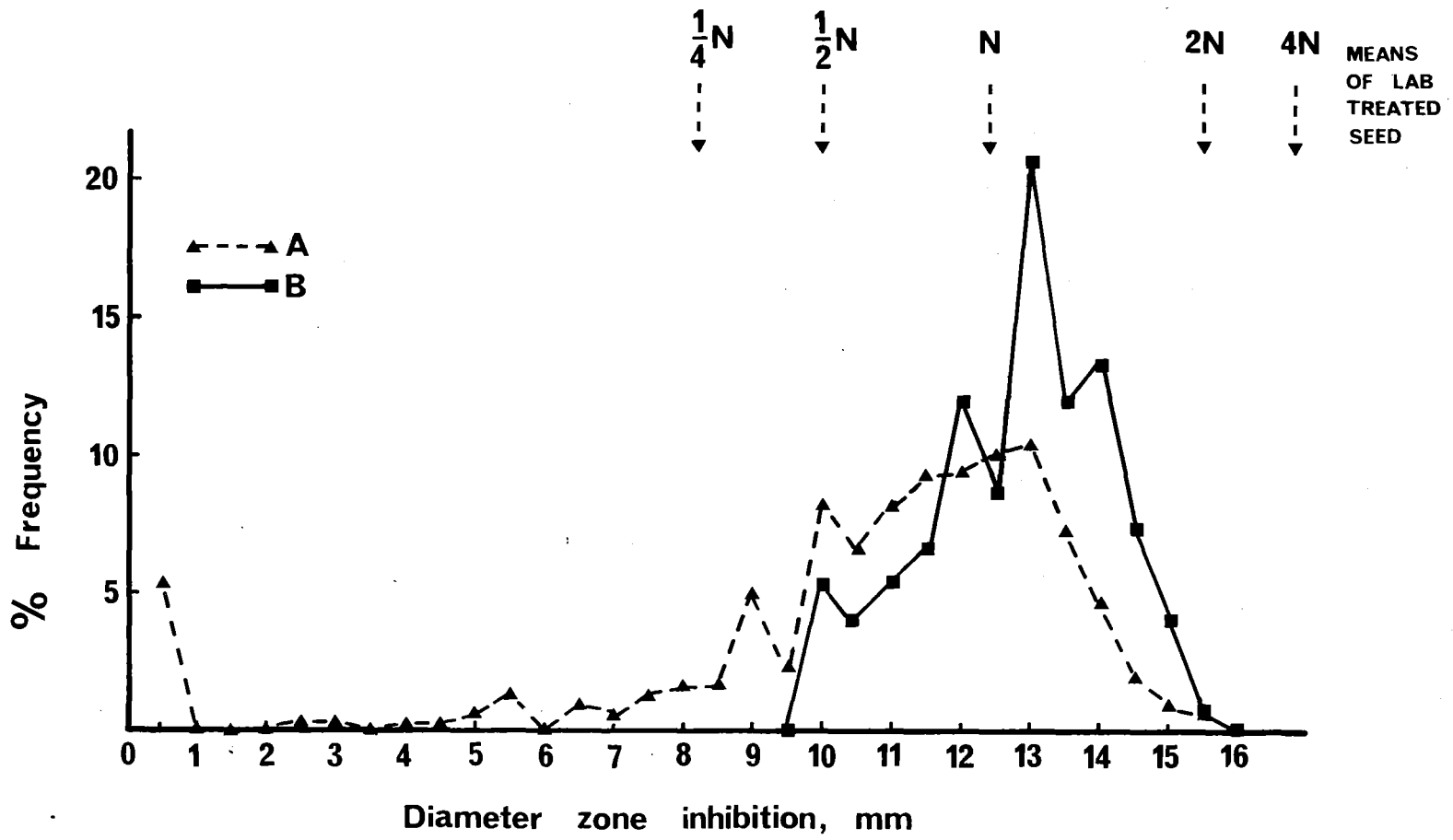


FIG. 17. Distribution of inhibition zone diameters of *A. niger* by captan applied commercially to wheat seed by two makes of slurry treater.



rate of captan. However, by this method, all seeds did receive at least half the recommended captan rate.

TABLE 18: Zones of inhibition of *A. niger* for standard captan treatments by seed and paper disc methods.

Rate captan	Method	Zone of inhibition (mm)			Std. deviation (mm)	% coefficient of variance
		Avg.	Min.	Max.		
¼N	seed	8.2	6.2	10.1	0.84	10.15
	disc	9.1	8.2	10.1	0.73	8.05
½N	seed	10.0	9.0	11.2	0.56	5.62
	disc	10.5	9.8	11.6	0.56	5.36
N	seed	12.4	10.0	13.9	0.91	7.38
	disc	12.9	10.9	15.8	1.25	9.65
2N	seed	15.5	13.1	16.9	0.75	4.85
	disc	15.3	12.7	18.5	1.97	12.64
4N	seed	16.8	15.0	18.9	0.83	4.93
	disc	17.0	13.2	19.6	1.74	10.22

There was a significant difference at the 1% level between the inhibition zones produced by machine type A and machine type B (Appendix 4), and this can also be seen by the differences in standard deviation and % coefficient of variance shown in Table 19. Seed treated by machine type B was more evenly treated at a rate closer to the one recommended than was seed treated by machine A.

TABLE 19: Zones of inhibition of *A. niger* by wheat seed slurry treated with captan by five merchants.

Seed Merchant	Line	Zone of inhibition (mm)			Std. deviation (mm)	% coefficient of variance
		Avg.	Min.	Max.		
1	a	9.4	0.0	15.0	3.1	29.2
	b	9.9	0.0	13.7	2.6	27.5
2	a	11.4	7.6	14.2	2.1	20.1
3	a	9.8	0.0	13.5	3.3	30.2
4	a	9.8	0.0	17.4	3.6	34.2
	b	12.3	6.4	14.8	1.9	17.8
5*	a	12.1	9.7	14.4	0.9	8.0
	b	12.0	9.6	15.1	1.1	9.4
	c	12.4	10.0	15.0	1.2	9.6

* Merchant 5 used type B slurry treater; merchants 1-4 used type A slurry treater.

10.5 DISCUSSION

A difference in the uniformity of seed treatment between the two slurry treaters, A and B, was obvious both from visual examination and from the results of the bioassay tests. Slurry application by machine type A produced seed which varied in coverage from no captan to over twice the recommended rate, with an average below the recommended rate. As similar results were obtained with samples from different companies, this

variation must be due to the machine and not the operator. As the bulk of Canterbury seed wheat is slurry treated by machine type A, the results indicate that nearly 30% of this wheat seed is receiving less than half the rate of fungicide recommended for disease control. However, the incidence of bunt in New Zealand has continued to be kept at a low level, and this suggests that although distribution of captan on seed wheat treated by machine type A is uneven, it is still basically effective. This in turn suggests that the amount of captan per seed recommended by the manufacturer for disease control, and used by the seed merchant is somewhat higher than is actually necessary for good control of bunt. If this is the case, then the results found in this work are not as severe as they seem, in that if the recommended rate (N) is higher than necessary, then seed which in these tests fell into the ½N to N coverage group is probably receiving an adequate coating of fungicide after all. However, the fact still remains that there are individual grains being certified as treated which have no fungicide cover at all.

The purpose of treating seed with fungicide is to ensure that sufficient fungicidal material is on the seed to provide the desired level of protection. In Canterbury seed stores, this is not being achieved, as approximately 30% of the treated wheat seed is receiving only low amounts of fungicide. Although it could be argued that the present coverage is sufficient, to eliminate a disease such as bunt, all seed wheat sown should have an effective cover of fungicide. This is also necessary to protect seed and seedlings against soil-borne

pathogenic fungi such as *Pythium* and *Fusarium* spp. To achieve this, either a change in slurry treating machine could be made, for example, from type A to type B, (which this work showed to provide a more even coverage at nearer the recommended rate than did type A) or perhaps slurry treater A could be modified so that better results could be obtained.

10.6 EFFECTS OF FUNGICIDE COVER ON GERMINATION

Examination of samples received from seed merchants showed that some wheat grains were heavily covered in fungicide (Table 17). An experiment tested whether coverage at this high rate had any adverse effects on germination of the seed. Seeds from the three coverage grades, described in section 10.2, and seeds slurry treated at the five rates used in the bioassay standard tests were placed on damp blotting paper in a Copenhagen germinator at 21^{o}C. After 10 days, percentage germination was assessed. One hundred seeds for each treatment were tested.

Results.

No significant difference in percentage germination were found between any of the samples tested (Appendix 4), although at twice and four times the recommended rate of captan coverage, there was a slight decrease. However, merchants samples very rarely had a coverage of more than twice the recommended rate, and so it can be concluded that the amount of fungicide on commercially slurry treated wheat seed would have no adverse effects on germination.

SECTION B 3: DISCUSSION

In New Zealand, bunt, or stinking smut of wheat, is at present controlled by seed disinfection, but has not been entirely eliminated from the wheat crop. The majority of crops are completely free of the disease, but there must be some crops that contain infected plants, as tests on 1973 crop samples showed an infection rate of approximately 9% of contaminated samples. It would appear that the situation regarding bunt in the New Zealand wheat crop has changed little since Blair (1950) found 17.4% and 8.9% infection in the 1947 and 1948 crops respectively. Fungicidal treatment of wheat seed obviously has given good control of the disease, but there must still be pockets of infection around the country where the disease has not been properly controlled. There have been occassional severe outbreaks reported over the last decade, and in each case, the seed wheat had not been treated with fungicide. These outbreaks tend to emphasise the importance of continuing to treat all seed wheat, for if this treatment was discontinued, the number of infected crops would increase rapidly.

Although it is important that all seed wheat be treated, it is also important that the fungicide treatment be such that each seed receives the desired level of protection. A visual examination of wheat seed slurry-treated by Canterbury seed merchants indicated that coverage of the seeds with fungicide was very variable. Bioassay tests showed that this was the case; some 30% of treated seed

wheat was receiving less than half the recommended rate of fungicide coverage, and approximately 6% of this wheat seed was receiving no treatment at all. The seed tested, although from four different merchants, (Table 19), was all treated by the same make of slurry treater, and the variability appeared to be due to the design of the machine rather than improper use by the operators. Wheat seed treated by another make of slurry treater proved to be much less variable in coverage (Table 19), with all seed treated having at least half the recommended rate of fungicide per seed, and the average for these samples was near the recommended rate (Fig. 17).

All seed wheat must be adequately treated with fungicide, not only to effectively control bunt, but to protect seedlings against soil-borne fungi. In Canterbury at present, all seed is not adequately treated. Seed merchants should seriously consider either modifications to their present seed treating machinery, or changing to another type of machine so that a more uniform seed coverage can be obtained. If farmers could be persuaded that all seed wheat must be treated, and if seed merchants ensured that all seed wheat was adequately treated, then bunt could well be eliminated from the New Zealand wheat crop.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to my supervisor, Dr R.C. Close and to Dr D. Gareth Jones for their guidance and helpful advice given willingly throughout this study.

My thanks also go to Professor A.P. Mulcock, the staff of the Agricultural Microbiology Department, Lincoln College, and the staff of Plant Diseases Division, D.S.I.R., Lincoln for advice and assistance in many ways; to the Canterbury New Zealand Seed Company Ltd, P.G. Ellis and Co. Ltd, V. Luisetti & Co. Ltd, Dalgety New Zealand Ltd, N.Z. Farmers' Co-op. Assn (Canterbury) Ltd, and Ispray Ltd, Nelson, for provision of treated wheat samples; to the N.Z. Wheat Research Institute, Christchurch, for assistance in obtaining wheat samples; to Miss C. Reay for assistance in preparation of figures; to the Visual Aids Department, Lincoln College, and Mr R. Goundrill for advice and assistance with photography; and to Miss C. Taylor for typing this thesis.

The author was assisted financially by a grant from the Canterbury branch of the N.Z. Grain, Seed and Produce Merchants' Association, for which I thank them.

Special thanks to my wife Jane, and her mother Mrs E. Leeburn, for without their tremendous encouragement and assistance, this thesis could not have been completed.

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APPENDICES

Appendix 1: FOLIAR SPRAY TRIAL

a) Yield of Aotea wheat from foliar spray trial ($g/0.5m^2$)

All treatments

benomyl (3 sprays)	154.9	a
mancozeb (2 sprays)	149.0	a
benomyl	140.7	a
thiophanate-methyl	139.3	a
untreated	130.7	a

S.E. Mean = 8.593

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Blocks	4	232932.031		
Treatments	4	173009.656	43252.414	1.17
Error	16	590794.000	36924.625	
Total	24	996735.688		

S.E. = 19.215 C.V. = 13.440

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b) Thousand grain weight of Aotea wheat from spraying trial

All treatments

benomyl (3 sprays)	42.5	a
mancozeb (2 sprays)	41.4	b
benomyl	41.2	b
thiophanate-methyl	39.5	b
untreated	39.1	b

S.E. Mean = 0.025

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Blocks	14	2469.179		
Treatments	4	12124.929	3031.232	30.54**
Error	56	5557.890	99.248	
Total	74	20152.000		
S.E. = 0.099		C.V. = 2.441		

c) Number of grains per ear of Aotea wheat from spraying trial

All treatments

benomyl (3 sprays)	29.3	a
mancozeb (2 sprays)	25.8	ab
benomyl	24.9	ab
thiophanate-methyl	23.7	b
untreated	23.6	b

S.E. Mean = 1.126

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Blocks	4	526046.250		
Treatments	4	1077019.126	269254.781	4.24*
Error	16	1015478.625	63467.414	
Total	24	2618544.002		
S.E. = 2.519		C.V. = 9.864		

d) Ear length (in cm) of Aotea wheat from foliar spray trial

All treatments

benomyl (3 sprays)	7.2	a
mancozeb (2 sprays)	6.8	b
benomyl	6.7	b
thiophanate-methyl	6.6	b
untreated	6.5	b

S.E. Mean = 0.875

Duncans test - nonsignificant groups at 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Blocks	4	5820.539		
Treatments	4	13767.742	3441.935	8.97**
Error	16	6137.867	383.616	
Total	24	25726.148		

S.E. = 1.958 C.V. = 2.877

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e) Plant height (in cm) of Aotea wheat from spraying trial

All treatments

benomyl (3 sprays)	58.4	a
mancozeb (2 sprays)	56.6	a
benomyl	56.2	a
thiophanate-methyl	55.2	a
untreated	55.4	a

S.E. Mean = 0.834

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Block	4	172695.000		
Treatments	4	289025.000	72256.250	2.07
Error	16	556844.000	34802.750	
Total	24	1018564.000		
S.E. = 1.865		C.V. = 3.304		

Appendix 2: WHEAT VARIETAL RESISTANCE TO *SEPTORIA* INFECTION

a) D.S.I.R. trial - % *S. tritici* on leaf 2 at G.S. 11.1

All treatments

Aotea	34.9	a
Cross 7	34.2	a
Gamenya	32.5	ab
Kopara	28.6	abc
Hilgendorf	24.9	bc
Arawa	23.3	c

S.E. Mean = 1.880

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	5	5987702.255	1197540.450	6.77**
Error	24	4244858.255	176869.093	
Total	29	10232560.511		
S.E. = 4.205		C.V. = 14.123		

b) Lincoln College trial - % *S. tritici* on leaf 2 at G.S. 11.1 x sowing date

Factors		Time of Sowing				
	B	March 0	April 1	May 2	June 3	
Varieties	A					
Aotea	0	37.35	36.41	35.89	43.14	38.20
Arawa	1	24.48	28.06	22.05	26.70	25.32
Hilgendorf	2	27.39	28.24	27.47	26.65	27.29
Kopara	3	24.99	22.20	25.30	26.51	24.75
Karamu	4	25.71	28.50	24.98	28.27	26.86
Mean		27.98	28.68	27.14	30.13	28.48

S.E. Mean A = 0.605 (variety)

S.E. Mean B = 0.541 (sowing date)

All treatments

4	Aotea	June	43.1	a
1	Aotea	March	37.3	b
2	Aotea	April	36.4	b
3	Aotea	May	35.8	b
18	Karamu	April	28.5	c
20	Karamu	June	28.2	c
10	Hilgendorf	April	28.2	c
6	Arawa	April	28.0	c
11	Hilgendorf	May	27.4	cd
9	Hilgendorf	March	27.3	cd
8	Arawa	June	26.7	cde
16	Kopara	June	26.5	cde
12	Hilgendorf	June	26.0	cde

17	Karamu	March	25.7	cde
15	Kopara	May	25.3	cde
13	Kopara	March	24.9	cde
19	Karamu	May	24.9	cde
5	Arawa	March	24.4	cde
14	Kopara	April	22.2	de
7	Arawa	May	22.0	e

S.E. Mean = 1.210

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Blocks	4	450933.000		
Treatments	19	28296626.523	1489296.132	20.34**
A	4	24475492.523	6118873.130	83.58**
B	3	1204416.000	401472.000	5.48**
AB	12	2616718.001	218059.833	2.97**
Remainder	0	0.000	0.000	0.00
Error	76	5563652.503	73205.954	
Total	99	34311212.031		
S.E. = 2.705		C.V. = 9.496		

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c) Numbers of perithecia (total of both *Leptosphaeria nodorum* and *Mycosphaerella* sp.) in stubble of Lincoln College trial (mean of 10 leaves obtained April, 1974.)

All treatments

Aotea	68.2	a
Hilgendorf	41.3	b
Arawa	35.0	b
Kopara	33.2	b
Karamu	33.2	b

S.E. Mean = 4.646

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	4	7130.600	1782.650	10.31**
Error	35	6046.375	172.753	
Total	39	13176.975		

S.E. = 13.143 C.V. = 31.127

Appendix 3: NUMBERS OF *TILLETIA CARIES* TELIOSPORES DETECTED

a) Number of teliospores per wheat variety bulk grain sample (mean no./field).

All treatments

Aotea	2.7	a
Arawa	0.5	b
Triple Dirk	0.5	b
Gamenya	0.5	b
Hilgendorf	0.2	b
Raven	0.2	b

S.E. Mean = 0.395

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	5	18.708	3.741	5.98**
Error	18	11.250	0.625	
Total	23	29.958		

S.E. = 0.790 C.V. = 99.861

b) Numbers of teliospores detected by the centrifuge and cellulose membrane method (mean 25 fields).

All treatments

Cellulose membrane	32.2	a
Centrifuge	29.8	a

S.E. Mean = 3.220

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	1	28.799	28.799	0.27
Error	18	1867.200	103.733	
Total	19	1896.000		

S.E. = 10.184 C.V. = 32.854

Appendix 4: WHEAT SEED SLURRY-TREATED WITH CAPTAN

a) Comparison of machine types - zone of inhibition of *A. niger* (mm) - mean 3 reps.

All treatments

Machine type B	12.4	a
Machine type A	9.9	b

S.E. Mean = 0.339

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	1	15475.360	15475.360	26.81**
Error	98	56560.200	577.144	
Total	99	72035.560		

S.E. = 2.402 C.V. = 21.377

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b) Germination of Aotea wheat seed slurry-treated with captan in the laboratory

All treatments	Mean Percent Germination	
untreated	85.0	a
½N	84.6	a
N	81.4	ab
¼N	81.3	ab
2N	77.6	b
4N	77.2	b

S.E. Mean = 1.445

Duncans test - nonsignificant groups 1% level

Analysis of Variance

Due to	DF	Sums of Sqs.	Mean Sq.	F
Treatments	5	3343740.002	668748.000	5.33**
Error	30	3763100.001	125436.666	
Total	35	7106840.003		
S.E. = 3.541		C.V. = 4.359		